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Breast Cancer

Innovations in Research and Management

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This book is dedicated to Umberto Veronesi, to his memory, and especially to his scientific and medical heritage.

Preface

The scientific and patient care communities have witnessed significant improvements in the diagnosis and treatment of breast cancer. Breast cancer is the leading cause of cancer morbidity and mortality in women worldwide. Screening, early diagnosis, and personalized treatments have provided better patient management, improved efficacy of therapies, and reduced mortality. Additional knowledge has been obtained by improving histopathological testing and conducting molecular and genetic investigations, which have also resulted in better therapies.

Progress in care of breast cancer patients has been achieved due to the clinical trials designed and conducted to demonstrate the efficacy and safety of therapies. This accumulation of evidence from randomized trials has resulted in a substantial improvement in patient care. Clinical trials can provide evidence indicating treatment efficacy but do not provide direct extrapolation on "how to treat the Individual Patient." The required intellectual step for extrapolation of useful details needed for adapting information from clinical trial results for the purpose of patient care is the multidisciplinary approach, a relatively novel methodology for discussion and negotiation involving several professional perspectives to define a common modality of diagnosis and treatment. With this spirit in mind, this book has been created, touching on all aspects of innovation in the care of patients with breast cancer. The editors and authors of each section and chapter are scholars in the field of breast cancer and are experts in conducting multidisciplinary discussions.

Professor Umberto Veronesi, who conceived the idea of this book to summarize modern developments in the diagnosis and treatment of breast cancer, was an internationally renowned innovator of diagnosis and all modalities of therapy for women with breast cancer. In the 1960s, he introduced the concepts that breast cancer is a disease with widespread extension of micrometastasis, and that the least extensive treatment (either surgical, radiation, or systemic) might suffice for obtaining the optimal therapeutic result. This approach involves specifically maintaining efficacy while reducing the burden of side effects of therapeutic and diagnostic interventions. Clinical research was conducted by him and others to intensively investigate this personalized approach for women with the disease. Areas of these clinical trial investigations included use of quadrantectomy instead of mastectomy, partial intraoperative radiation therapy instead of whole breast irradiation, sentinel node biopsy instead of full axillary dissection, and assigning systemic therapies according to features that predict responsiveness to different treatments instead of using the "same therapy for all" approach. His dedication to prevention was also methodologically remarkable, from pioneering work in early diagnosis to investigating chemoprevention in clinical trials. This book is a comprehensive presentation of breast cancer research and treatment, describing past and present information and including thoughts about the future.

Professor Umberto Veronesi passed away on November 8, 2016. Although he did not survive to see the book's birth, he was intensively involved in the editing until the last days of his life. He remained an example for all of us, insisting that the work should go on. This book is dedicated to him, to his memory, and especially to his scientific and medical heritage.

Special appreciation is extended by all editors and authors to Mrs. Lucia Racca, the backbone of Professor Veronesi's office for many years, who maintained the coordination of the editorial office until the completion of the work, well beyond her retirement.

This book is an important resource covering all aspects of breast cancer carcinogenesis, prevention, diagnosis, and surgical, radiation, and systemic therapies. It is particularly suited for those who seek exposure to a broad spectrum of knowledge of a multidisciplinary approach to understand the disease and facilitate optimal patient care.

Milan, Italy
September 2017

Aron Goldhirsch

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An Integrated View of Breast Cancer Biology

Fundamental Pathways in Breast Cancer 1: Signaling from the Membrane

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Yekaterina Poloz, Ryan J.O. Dowling, and Vuk Stambolic

1.1 Introduction

Breast cancer (BC) is the most frequently diagnosed cancer in women worldwide, with 1.7 million new cases diagnosed in 2012 [1]. In the United States alone, 231,840 new cases and 40,290 related deaths are expected to be seen in 2015 [2]. This heterogeneous disease is classified into several molecular subtypes, depending on the presence of specific cell surface receptors, including the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor 2 receptor (HER2) [3]. Luminal A BCs are ER^+ and/or PR^+ but $HER2^-$. These are the most commonly seen BCs and have the best prognosis. Luminal B BCs are ER^+ and/or PR^+ and sometimes $HER2^+$. These tumors have a higher proliferative index and are more aggressive. $HER2^+$ BCs are ER^- and PR^- but $HER2^+$. This subtype usually presents at a younger age with a poorer tumor grade and lymph node involvement, but the prognosis has improved dramatically since the clinical implementation of Herceptin, an anti-HER2 antibody. About 20% of BCs are triple negative (TNBC) or basal-like, that is, they are ER^-, PR^-, and $HER2^-$. These tumors are often aggressive, have poorer prognosis, and lack any targeted therapies.

Research has focused extensively on the role of cell surface receptors like HER2 in the pathobiology of BC. There are numerous families of cell surface receptors, like receptor tyrosine kinases (RTKs), one example being HER2, and G protein-coupled receptors, that sense extracellular cues and transmit them into intracellular messages that regulate cell growth, proliferation, survival, migration, and differentiation. These receptors are often deregulated in BC and lead to tumor growth and metastasis. This chapter will focus on the identification of the receptors most often deregulated in BC, the common signaling pathways they activate, and the crosstalk that links them to one another.

1.2 RTKs and Their Downstream Signaling Targets

RTKs are cell surface receptors found on a diversity of cell types. All RTKs comprise an N-terminal extracellular ligand-binding domain, a single-pass transmembrane domain, and a C-terminal tyrosine kinase domain. Ligand binding induces a conformational change leading to the receptor homo- or heterodimerization and the consequent autophosphorylation of a series of tyrosine residues in the C-terminal tail. The phosphorylated tyrosines then act as docking sites for the SRC homology 2 (SH2) and phosphotyrosine-binding (PTB) domain-containing proteins, many of which are shared by the different RTKs. The RTK signaling program converges on the two major signaling pathways, namely, the phosphoinositide 3-kinase-protein kinase B/AKT (PI3K-PKB/AKT) and the rat sarcoma-mitogen-activated protein kinase/ERK (Ras-MAPK/ERK), that go on to regulate critical cellular processes like cell growth, proliferation, differentiation, migration, and apoptosis (Fig. 1.1).

One of the SH2 domain-containing effectors of RTKs is the regulatory subunit of the class I PI3K (p85), which when bound to the activated RTK or one of its tyrosine phosphorylated adaptors relieves its inhibition of the p110 catalytic subunit of PI3K, thereby leading to its activation [4]. PI3K p110 then phosphorylates a resident membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP2), to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3), a major lipid

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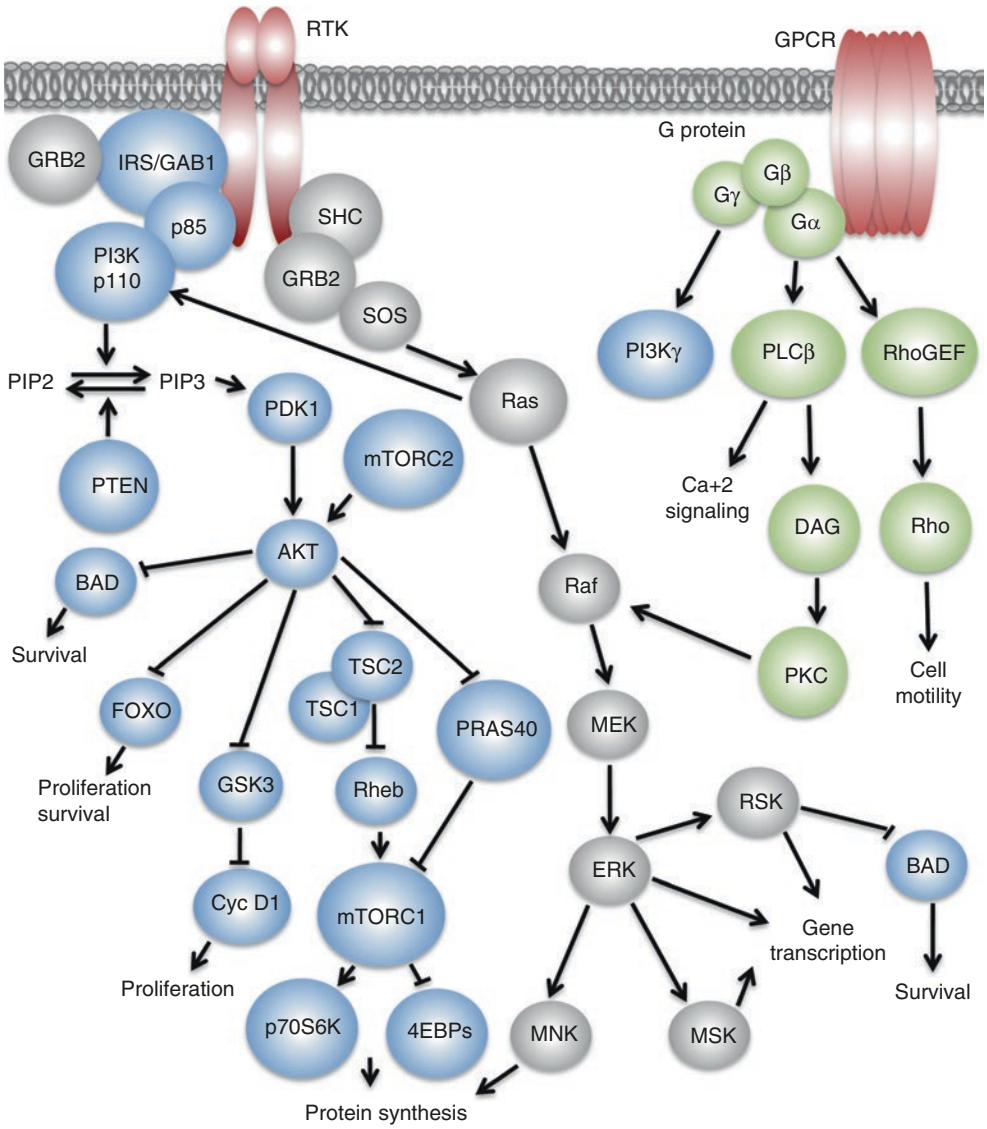

Fig. 1.1 RTK and GPCR signaling networks

second messenger [5]. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) counteracts the action of PI3K and converts PIP3 back to PIP2 [6, 7]. PIP3 recruits the pleckstrin homology (PH) domain-containing proteins to the membrane, of which there are more than 250 in the human genome, including AKT and the 3-phosphoinositide-dependent protein kinase 1 (PDK1) [8]. AKT is phosphorylated by PDK1 on threonine 308 (T^{308}) and consequently by the mammalian target of rapamycin complex 2 (mTORC2) on serine 473 (S^{473}), leading to its full activation [9, 10]. AKT then activates the mammalian target of rapamycin complex 1 (mTORC1) through two distinct pathways. AKT phosphorylates and suppresses the GTPase-activating protein (GAP) activity of the tuberous sclerosis complex 2 (TSC2) toward the Ras homolog enriched in the brain (Rheb) [11–13]. On the other hand, AKT phosphorylates and inhibits proline-rich AKT substrate of 40 KDa (PRAS40), which is implicated in the regulation of mTORC1 [14, 15]. mTORC1 regulates cell growth by controlling mRNA translation, via direct phosphorylation of the S6 kinase (p70S6K) and the 4E binding proteins (4EBPs) [16, 17]. AKT also phosphorylates the forkhead box O transcription factors (FOXOs), which results in their nuclear exclusion and proteasomal degradation, thus releasing cells from the FOXO-mediated cell cycle arrest [18–20]. The deactivation of FOXO, along with another target of AKT, the B-cell lymphoma 2 (BCL2)-associated agonist of cell death (BAD), coordinately represses apoptosis [21]. Finally, the AKT-mediated inhibition of glycogen synthase kinase 3 (GSK3) inhibits nuclear export and proteasomal degradation of cyclin D1, thus leading to its nuclear accumulation and induction of cell proliferation [22]. Thus, the PI3K-AKT pathway mainly regulates the cellular growth, proliferation, and survival programs in cells. Other than RTK deregulation, activating mutations in PI3K or deletion of PTEN is often found in BCs and further drives the oncogenic program in cells [23].

The Ras-ERK pathway is the other major signaling network that is modulated by the RTKs. The Src homology 2 domain-containing (SHC) and the growth factor receptor bound 2 (GRB2) are the main adaptor proteins that link the activated RTKs to the Ras-ERK pathway [24]. The RTKs interact with and activate SHC directly, which then recruits GRB2 to the cell membrane. Alternatively, GRB2 can also interact with RTKs directly or through another adaptor protein, like one of the insulin receptor substrates (IRSs) [25, 26]. GRB2 associates with the son of sevenless (SOS), which then recruits and activates Ras, by acting as a guanine nucleotide exchange factor (GEF), converting the GDP-bound Ras into the active GTP-bound form [27]. In a sequential manner, Ras activates Raf, which phosphorylates and activates MEK, which in turn phosphorylates and activates ERK [28, 29]. ERK is a serine-threonine kinase that has hundreds of cytoplasmic and nuclear targets. For example, ERK translocate to the nucleus and activates transcription factors like Ets-like gene 1 (Elk1) and c-Myc [30, 31]. In the nucleus, ERK also activates the mitogen- and stress-activated protein kinases (MSKs), which activate transcription factors like the cyclic AMP-responsive element-binding protein (CREB) and the activating transcription factor 1 (ATF1) [32]. In the cytoplasm, ERK phosphorylates the p90 ribosomal S6 kinases (RSKs), which inhibit apoptosis by phosphorylating BAD, but also translocate to the nucleus and activate transcription factors like CREB and c-Fos [33–36]. ERK-mediated phosphorylation of the MAPK-interacting kinases (MNKs) induces mRNA translation through phosphorylation of the eukaryotic initiation factor 4E (eIF4E) [37, 38]. The Ras-ERK pathway thus controls diverse cellular processes including cell growth, proliferation, differentiation, migration, and apoptosis.

In addition to the parallel activation immediately downstream of the receptors, coordination between the PI3K-AKT and the Ras-ERK signaling pathways can also be achieved by the interaction of activated Ras with the PI3K p110 catalytic subunit, independently of p85, leading to the PI3K-AKT pathway activation (Fig. 1.2) [39]. Like AKT, ERK and RSK can also phosphorylate and inhibit TSC2, leading to mTORC1 activation [40, 41]. The two pathways are also subject to the multiple levels of feedback inhibition. MEK promotes the membrane localization of PTEN, which downregulates the PI3K-AKT signaling pathway, while AKT can phosphorylate and inhibit Raf [42–44]. Furthermore, mTORC1, S6K, and ERK can downregulate both pathways by phosphorylating RTK substrates, like IRS1, on multiple inhibitory serine residues [45–47]. Thus, multiple feedback loops and crosstalk between the PI3K-AKT and the Ras-ERK pathways orchestrate the dynamic and intricate, context-dependent effects of multiple growth factors through their cognate RTKs.

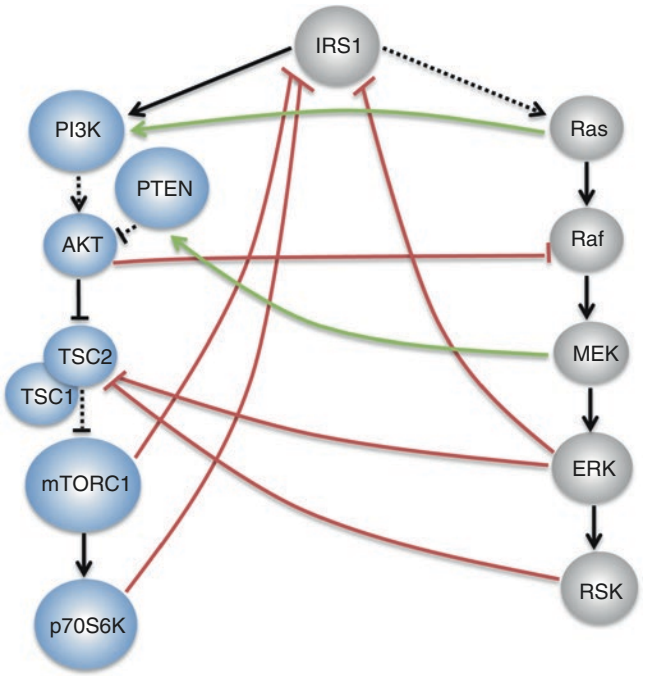

Fig. 1.2 Crosstalk between the PI3K-AKT and the Ras-ERK signaling pathways. *Green lines* indicate activation and *red lines* indicate inhibition. *Solid black lines* indicate a direct interaction, while *dashed black lines* indicate an indirect interaction

1.3 RTKs Often Deregulated in BC

The deregulation of RTK signaling plays an important role in the pathophysiology of many cancers, including BC [48]. Several mechanisms lead to the deregulation of RTK signaling, including RTK gene amplifications, activating mutations, protein overexpression, ligand overexpression or hyperactivation, and crosstalk with other cellular signaling components. Members of the ERBB family, MET, the insulin receptor (INSR), and the insulin-like growth factor receptor (IGF1R) are RTKs that are most often deregulated in BC.

1.3.1 HER2

The amplification of the *HER2* gene, a member of the ERBB family of RTKs, is seen in approximately 20% of BCs, and HER2 overexpression correlates with a worse BC prognosis [49–51]. In these patients, HER2 overexpression correlates with tumor size, grade, proliferative index, aneuploidy, lack of steroid hormone receptors, and metastatic disease. HER2 (also named ERBB2 or NEU), belongs to the ERBB family, with three additional members: the epidermal growth factor receptor (EGFR, also named ERBB1), ERBB3, and ERBB4, all of which have been shown to be overexpressed and/or hyperactivated in BC to varying degrees. For example, EGFR is often overexpressed in basal-like TNBC [52].

There are 11 ligands that activate this family of RTKs, and they can be subdivided into three groups [53]. The first includes the epidermal growth factor (EGF), the transforming growth factor α (TGFα), and amphiregulin, which bind specifically to EGFR. The second includes betacellulin, heparin-binding EGF (HB-EGF), and epiregulin, which bind EGFR and ERBB4. Neuregulins (NRGs) make up the third group of ligands and are further subdivided into two subgroups, based on the ability to activate ERBB3 and ERBB4 (NRG1 and NRG2) or ERBB4 alone (NRG3 and NRG4). All ligands exist as membrane-anchored precursors, often co-expressed and even overexpressed with the ERBBs in the same cancer cells. Metalloproteases, mainly of the a disintegrin and metalloprotease (ADAM) family, cleave the precursors, leading to ectodomain shedding and activation of ERBB signaling in an autocrine or paracrine fashion [54].

Like other prototypical RTKs, all ERBBs can form functional homo- or heterodimers, with the exception of HER2, which does not appear to bind a ligand, and ERBB3, which is impaired in the intrinsic kinase activity and thus cannot form functional homodimers [55, 56]. Though HER2 is not self-autonomous, its extracellular domain conformation mimics that of the ligand-bound receptor, thus allowing HER2 to form functional heterodimers with other ERBBs [57]. HER2 is in fact the preferred binding partner of other ERBBs, and intriguingly the HER2-ERBB3 heterodimer is the most mitogenic and transforming of all the receptor combinations [58–61]. The C terminus of each of the ERBBs is unique (11–25% identity) and is able to bind to a diversity of intracellular targets. All of the ERBB members activate the Ras-ERK signaling pathway by directly interacting with the adaptor proteins SHC and GRB2 [62]. The regulatory subunit of PI3K (p85) directly interacts with ERBB3 and ERBB4. ERBB3 has the most [6] binding sites for p85, while EGFR and HER2 lack them all together, thus the HER2-ERBB3 heterodimer is the most potent activator of the PI3K-AKT signaling pathway, promoting cell growth, proliferation, and survival [63, 64]. Alternatively, ERBBs can activate the PI3K pathway through Ras. Together with the multitude of ligands, the different combinations of receptor dimers, and the unique C-terminal tails, this family of RTKs is capable of regulating diverse cellular processes implicated in cell growth, proliferation, differentiation, migration, and apoptosis.

1.3.2 MET

The hepatocyte growth factor receptor or MET is another RTK that is overexpressed in about 20% of BCs, particularly in the basal-like TNBCs [65]. Hepatocyte growth factor (HGF) is the only known ligand of MET, and it is often co-expressed with its receptor in the same tumor cells, particularly in the leading edge of the tumor [66, 67]. The expression of both, the receptor and the ligand, correlates with tumor grade, proliferative index, metastatic disease, and poor prognosis [68–74]. The HGF-mediated activation of MET leads to activation of the Ras-ERK pathway through the direct interaction of SHC and GRB2 with the receptor or through the recruitment of an insulin-like substrate (IRS)-like adaptor, the GRB2-associated-binding protein 1 (GAB1). The p85 regulatory subunit of PI3K also interacts with MET directly or through GAB1 and leads to activation of the PI3K-AKT pathway [75].

1.3.3 INSR

The INSR is overexpressed in as many as 80% of BCs and is associated with poor survival [76, 77]. The INSR is encoded by a gene composed of 22 exons found on chromosome 19. From this single gene, two receptor isoforms, INSR-A and INSR-B, are expressed as a result of alternative splicing. These two isoforms differ in inclusion/exclusion of exon 11, a 36 bp region encoding a 12 amino acid peptide located at the C-terminal end of the INSR alpha subunit [78]. INSR-B represents the full-length isoform and is expressed in insulin-responsive tissues including the liver, muscle, and adipose tissue. Conversely, INSR-A is expressed from the spliced transcript that lacks exon 11 and plays a significant role in fetal development by regulating cell growth and proliferation [79, 80]. The INSR-A and INSR-B isoforms display unique ligand specificity and downstream signaling potential. INSR-A exhibits an almost twofold higher affinity for insulin as compared to INSR-B and has a much stronger affinity for the insulin-like growth factor II (IGFII) [81–83]. INSR-A is the prevailing isoform overexpressed in both BC cells in culture and patient tumors [77, 84]. Therefore, increased INSR-A expression may negatively impact BC development, particularly in the context of hyperinsulinemia, as in the cases of diabetes or obesity. Indeed, hyperinsulinemia is an adverse prognostic factor in BC that is associated with increased risk of recurrence or death [85]. INSR-A expression is also elevated beyond that of the related IGF1R in some BCs suggesting INSR-A plays a role in mediating the growth-promoting effects of IGF-II in breast tumorigenesis [84, 86].

On the cell surface, the INSR exists as a heterotetrameric protein comprised of two extracellular alpha subunits and two transmembrane beta subunits. The beta subunit of the receptor possesses tyrosine kinase activity, which is stimulated upon binding of the ligand to the alpha subunit [87, 88]. Upon activation, INSR phosphorylates a number of substrates including IRS1–4, SHC, and GAB1 [89]. IRSs and GAB1 recruit the p85 regulatory subunit of PI3K, leading to

the PI3K-AKT pathway activation. The Ras-ERK pathway is activated by the recruitment of GRB2-SOS complex by SHC or IRSs [26, 90]. INSR-B regulates the metabolic effects of insulin mainly through the PI3K-AKT pathway, while INSR-A activates the mitogenic program through both, the Ras-ERK and the PI3K-AKT pathways [91, 92]. Consequently, inhibition of INSR-A is actively being explored as a therapeutic option in breast and other cancers with clinical trials focusing on testing small molecule inhibitors and monoclonal antibodies directed against key components of these signaling networks [93]. Systemic modification of receptor ligands represents another strategy for targeting INSR signaling in cancer. For example, reduction in circulating insulin levels via administration of the antidiabetic drug metformin is being explored as a treatment option for cancers associated with obesity and hyperinsulinemia, especially BC. Indeed, administration of metformin to early-stage, nondiabetic BC patients led to reductions in circulating insulin and cancer cell proliferation, as well as suppressed INSR activity as indicated by reductions in AKT and ERK signaling [94].

1.3.4 IGF1R

Close to 50% of human breast tumors express the activated form of IGF1R, and gene expression signatures consistent with IGF1R activation are associated with poor outcome in BC patients [95, 96]. The IGF1R is homologous to the INSR but exhibits preferential binding to IGFI and IGFII over insulin. It is also a heterotetrameric protein complex consisting of two extraceulluar alpha subunits and two transmembrane beta subunits but plays a more significant role in the regulation of mitogenic signaling. The IGF1R shares numerous binding partners and effector proteins with the INSR, including IRSs and SHC adaptors, and is known to stimulate cell growth and proliferation via activation of the PI3K-AKT and Ras-ERK signaling pathways [93, 97]. A second IGF receptor, namely, IGF2R, is also commonly expressed by numerous cells; however, it lacks catalytic activity and is not involved in intracellular signaling [98]. Instead, IGF2R exhibits a high affinity for IGFII and is thought to sequester the growth factor from stimulating IGF1R [97, 99]. As a result, IGF2R may exhibit tumor suppressor properties by decreasing the bioactivity of IGFII and indirectly modulating signaling by IGF1R.

Due to their homology and strong structural similarities, the INSR and IGF1R have the ability to form hybrid receptors composed of one hemireceptor of each type. In addition, the two INSR isoforms can also combine to form hybrids, generating the potential for multiple insulin and IGF-sensitive receptors (INSR-A, INSR-B, INSR-A/B, IGF1R, INSR/IGF1R) to be expressed by a single cell. Hybrid receptors appear to bind IGFI with a higher affinity than insulin, and they exhibit different ligand specificities depending on the INSR isoform present. For example, INSR-A/IGF1R hybrids bind IGFI, IGFII, and insulin, while INSR-B/IGF1R hybrids typically bind IGF1 [91]. Since cancer cells frequently express high levels of both INSR and IGF1R, it is not surprising that they also overexpress hybrid receptors. Indeed, human breast tumors express high levels of hybrid receptors, and most of the effects of IGF1 are believed to be mediated by INSR-A/IGF1R hybrids. Furthermore, BC cells are known to secrete IGFII, creating the potential for autocrine stimulation of tumor cell growth and proliferation via activation of INSR-A, IGF1R, and INSR/IGF1R hybrid receptors [84, 100]. Consequently, human BC cells are highly sensitive to the growth-promoting effects of insulin and IGFs, and INSR/IGF1R expression may be a key event in tumor development and growth.

1.4 GPCRs and Their Downstream Signaling Targets

The G protein-coupled receptors (GPCRs) are the largest group of cell surface receptors that regulate cell motility, growth, proliferation, differentiation, and survival. The discovery of the Mas oncogene, a GPCR, in 1986 provided the first direct link between GPCRs and their role in cellular transformation [101]. Since then, many GPCRs where shown to be overexpressed or mutated in a diversity of cancers, including BC.

GPCRs are seven-pass transmembrane domain-containing receptors with an intracellular C-terminal tail that interacts with the heterotrimeric G proteins [102]. Upon ligand binding, the receptor undergoes a conformational change that allows it to act as a GEF, converting the GDP-bound G protein α subunit to the GTP-bound, active form. This causes the G protein α subunit to dissociate from the βγ subunits, initiating a multitude of signaling cascades. There are numerous G protein subtypes, each with unique signaling abilities. For example, the $G\alpha_{12/13}$ activates several Rho GEFs leading to activation of Rho, a small GTPase that regulates cytoskeletal dynamics and cell motility, largely implicated in cancer metastasis. $G\alpha_{q/11}$ activates the phospholipase C beta (PLCβ), which initiates the calcium and diacylglycerol (DAG) signaling cascades that regulate cell motility, proliferation, and gene expression. The GPCR signaling also crosstalks to the PI3K-AKT and the RAS-ERK pathways. The DAG-activated protein kinase C (PKC) phosphorylates and activates Raf, thereby leading to the activation of ERK [103]. The Gβγ subunits bind directly to PI3Kγ and activate the PI3K-AKT signaling pathway [104].

1.5 GPCRs Often Deregulated in BC

1.5.1 PAR1

The protease activated receptor 1 (PAR1) is a GPCR that is overexpressed in TNBC and correlates with metastatic disease and poor prognosis [105, 106]. The zinc-dependent matrix metalloprotease 1 (MMP1), thrombin, and other proteases cleave the extracellular domain of PAR1 exposing a new N terminus that binds to and activates the receptor [107, 108]. PAR1 then couples to multiple G proteins ($G\alpha_{q/11}$, $G\alpha_{i/o}$, $G\alpha_{12/13}$) to regulate cell migration and proliferation, in part through the activation of Rho and ERK, respectively. PAR1 has been shown to be required and sufficient for the regulation of growth and invasion of BC cells in a mouse xenograft model [107, 109].

1.5.2 GPR161

The GPR161 is another GPCR that is overexpressed in TNBC and correlates with cancer relapse [110]. Overexpression of GPR161 in mammary epithelial cells transforms them via a yet unidentified mTORC1-dependent signaling pathway [110].

1.5.3 Wnt

The Wnt signaling pathway is hyperactivated in basal-like BCs and correlates with poor survival [111, 112]. The canonical Wnt signaling pathway results in nuclear accumulation of β-catenin, where it acts as a transcriptional coactivator for the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors [113]. In the absence of the Wnt signal, β-catenin is sequestered in the cytoplasm by a destruction complex, containing GSK3β, which targets β-catenin for proteasomal degradation. Frizzled (FZD) is the GPCR for the Wnt family of ligands. When FZD is activated by the Wnt ligand, it acts together with the co-receptors, the low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6), to disrupt the β-catenin destruction complex. This allows β-catenin to accumulate in the cytoplasm and translocate to the nucleus to activate its transcriptional program. The knockdown of FZD7 in TNBC cell lines reduces expression of β-catenin target genes, the transformation of these cells *in vitro*, and their ability to form tumors *in vivo* [114]. In addition, more than 40% of invasive breast tumors have a hypermethylation of the promoter, and therefore a strong downregulation of expression of the secreted frizzled-related proteins (sFRPs), the negative regulators of the Wnt signaling pathway [115, 116].

1.6 Crosstalk Between RTKs and GPCRs

The RTKs crosstalk with each other through multiple feedback and transactivation mechanisms. For example, MET can interact with and be transactivated by ERBBs, thus synergizing in the regulation of the downstream pathway components [117, 118]. Furthermore, RTK signaling often parallels or synergizes with GPCR signaling. The GPCRs can be upstream or downstream of the RTKs, and GPCRs are under the transcriptional regulation of RTKs and vice versa [119]. Furthermore, GPCRs and RTKs can transactivate each other. For example, GPCR activation regulates ectodomain shedding of the ERBB ligands. The PAR1 and the Wnt pathway have been shown to transactivate EGFR and HER2 in this manner [120–123]. Thus, GPCRs can activate the PI3K-AKT and the Ras-ERK pathways directly or through transactivation of the RTKs. In addition, EGFR-mediated activation of ERK induces nuclear translocation of the pyruvate kinase (PKM), which regulates β-catenin transcriptional activity [124]. The expression of β-catenin target genes can further be induced through the AKT- or RSK-dependent inhibition of GSK3β or the direct phosphorylation of β-catenin by AKT [125–127].

1.7 Other Receptors Deregulated in BC

The tumor microenvironment is a complex milieu of cell surface and secreted factors that affect BC development and progression. Tumor-associated fibroblasts (TAFs), endothelial cells, and inflammatory cells comprise the majority of the tumor microenvironment and express factors that affect tumor progression. Tumor cells express a number of non-RTK and/or non-GPCR receptors, the discussion of which is beyond the scope of this chapter, that sense signals from the microenvironment and often integrate them into the common pathways described above. For example, plexins, the receptors of semaphorins, originally described for their role in axon guidance, have now been implicated in BC metastasis, in part due to their ability to be transactivated by HER2 and MET and to activate Rho signaling [128, 129]. Tumor cells also express a number of cytokine receptors that interpret the pro-inflammatory signaling from leukocytes, tumor-associated macrophages (TAMs), TAFs, and autocrine loops. Cytokine receptors can activate several pro-survival and proliferation pathways but can also transactivate RTKs [130]. Lastly, integrins and cadherins, the cell adhesion mediators, are often deregulated in metastatic BC and play a central role in the epithelial-to-mesenchymal transition as well as in the activation of oncogenic signaling. Integrins can feed into both the PI3K-AKT and the Ras-ERK signaling pathways [131, 132]. Integrins also regulate ERBB expression at the mRNA translation level, as well as interact directly with

ERBBs and regulate their tyrosine kinase activity [133, 134]. Further insight into the complexity of these intracellular crosstalk networks will aid in the identification of effective therapeutic targets and the mechanisms of therapeutic resistance.

1.8 Outlook

BC is one of the most common cancers worldwide and the second leading cause of cancer-related death in women [135]. It is a heterogeneous disease with a complex molecular etiology. A great deal of research has focused on the mechanisms underlying BC development, growth, and progression. Dysregulated RTK signaling has been identified as a critical event in breast tumorigenesis. For example, HER2 and IR are overexpressed by 20 and 80% of BCs respectively, and mutation of PI3K, a key mediator of RTK signaling, is mutated in 35% of human breast tumors [77, 135, 136]. Identification of such oncogenic proteins has led to a deeper understanding of BC and allowed for the development of targeted therapies for the treatment of this disease. Nevertheless, additional research is required to characterize mechanisms of tumor initiation as well as therapeutic resistance. Indeed, crosstalk between different RTK pathways and the existence of signaling feedback mechanisms are poorly understood processes that play critical roles in BC development and resistance to therapy. In the future, fundamental studies focusing on these issues *in vitro* should be combined with clinical research and early phase clinical trials to further characterize the role of RTKs in BC and identify new targets for anticancer therapies.

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Fundamental Pathways in Breast Cancer 2: Maintenance of Genomic Stability

2

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2.1 Introduction

Mammalian cells preserve their genomic integrity by counteracting DNA damage [1]. DNA damage can originate from exogenous and endogenous insults. Exogenous insults involve environmental stress that originates from exposure to chemicals, UV light, tobacco smoke, chemotherapy, and radiotherapy. Endogenous DNA damage can arise from impaired DNA metabolic processes, intracellular oxidative stress, and oncogene activation. Cells respond to DNA damage by activating sensors, transducers, and effectors that jointly coordinate the so-called DNA damage response (DDR). When the amount of DNA damage is manageable, DDR activates cell cycle checkpoints that arrest cell cycle progression and allow DNA repair to correct the lesion thus preventing replication of damaged DNA. When DNA damage reaches levels beyond repair, cells activate self-destruction mechanisms that include apoptosis, autophagy, senescence, and necrosis.

There are five different mechanisms of repair designed for specific types of DNA lesions [2]:

- Base excision repair (BER) removes base damage. BER is mainly involved in the surveillance, recognition, and repair of oxidative DNA.
- Mismatch repair (MMR) corrects replicative errors and mismatched base pairs caused by faulty proofreading of DNA polymerases. MMR ensures low mutation rates in replicating cells.
- Nucleotide excision repair (NER) operates on a spectrum of helix-destabilizing bulky DNA lesions (global genome NER or GG-NER) or eliminates lesions in the transcribed strand of active genes (transcription-coupled NER or TC-NER). For example, NER removes bulky DNA adducts caused by exposure to various chemicals, alkylating agents, and UV radiation.
- Single-strand break (SSB) DNA repair corrects DNA breaks on one strand of the DNA double helix arising directly on the deoxyribose moieties or indirectly as intermediates of BER. SSBs are among the most frequent DNA lesions and are major threats to genetic stability and cell survival.
- Double-strand break (DSB) DNA repair resolves lesions that appear on both DNA strands. The DSB is the principle lesion deriving from ionizing radiation and radiomimetic chemicals. It is also caused when a replicative DNA polymerase encounters a DNA single-strand break or other type of DNA lesions. DSBs are intermediates in various biological events, such as V(D)J recombination in differentiating B cells. There are two major pathways of DSB repair: homologous recombination (HR) and nonhomologous end joining (NHEJ). During HR, the repair of the damaged strand occurs by retrieving genetic information from the undamaged complementary DNA sister strand. In contrast, NHEJ brings about the ligation of two DNA DSBs without the requirement for sequence homology between the DNA ends.

Although these mechanisms are tightly regulated, DNA repair defects occur thereby promoting the acquisition of mutations. Genomic instability results from a high frequency of DNA mutations ranging from nucleotide changes to chromosomal translocation, and it is a common feature of many cancers. De novo genetic alterations can initiate tumorigenesis, augment aggressiveness, and ultimately affect the overall prognosis of cancer patients. Recent studies have shown that different tumors have specific DNA repair defect signatures that involve more than one repair pathway [3]. A more comprehensive analysis of these signatures has shown that DNA repair mechanisms are not parallel distinct entities but are intimately interconnected and can influence each other. While a normal cell coordinates DNA repair to preserve its genomic integrity, cancer cells modify DNA repair hubs to

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maintain genomic instability without compromising survival. In fact, cancer cells are subjected to many stressful conditions including nutrient starvation, oxidative stress, hypoxia, chemotherapy, and radiation that ultimately result in genotoxic damage. Therefore, a cancer cell must rely on a certain level of functional DNA repair to cope with these additional sources of damage.

Regulation of DDR and DNA repair has a critical role in the development and treatment of breast cancer. Expression and genomic profiling studies have shown that breast cancer is a very heterogeneous disease [4]. There are different subtypes of breast cancers, and each type exhibits a range of biological and clinical behaviors (discussed more in detail in Chap. 1.4). Luminal subtypes A and B both express markers of the luminal epithelial layer of normal breast tissue such as keratins 8/18 and are estrogen receptor (ER) positive. The ERBB2 subtype is characterized by the amplification and overexpression of ERBB2 (HER2) and neighboring genes at 17q12-q21 locus. The basal-like subtype expresses markers of the basal epithelial layer of normal breast tissue such as keratins 5/6. Cancer cells of this subtype do not express ER, progesterone receptor (PR), and HER2 and are therefore referred to as triple negative (TN). Other TN types include "normal-like" and the recently described "claudin-low" subtype, showing that most but not all TN are basal-like cancers. There are two main hypotheses that can explain the existence of different subtypes: (1) each subtype arises from a distinct tumor-initiating cell and (2) all subtypes share a common cell of origin that eventually acquired different somatic DNA mutations leading to different biological phenotypes. Although a definitive answer is lacking and both phenomena can coexist, it is clear that breast cancer subtypes are the results of distinct evolutionary processes. The genomic profile of breast cancers spans from a relatively simple landscape in luminal A subtype to a highly complex scenario in basal-like/TN subtype (Fig. 2.1). In this chapter, we will consider the role of genomic instability underlying the different biological and clinical features of each breast cancer subtype.

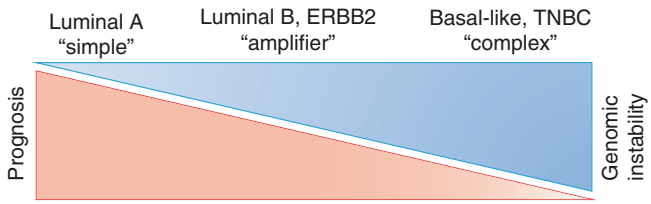

Fig. 2.1 Correlation between tumor aggressiveness and genomic instability in the different subtypes of breast cancer. Luminal A subtype tends to have good prognosis and a "simple" genomic landscape with few genetic alterations. Luminal B and ERBB2 subtypes have an "amplifier" genome with high frequency of gene duplications. As a consequence, their prognosis is less favorable than luminal A. Basal-like and TN breast cancers have a highly unstable "complex" genomic profile and have very poor prognosis

2.2 Genomic Instability in Luminal A/B Subtypes of Breast Cancers

Luminal A and B subtypes share similar biological characteristics. However, luminal B tumors are more genetically unstable and highly proliferative and have less favorable prognosis. Luminal A subtype tumors have few chromosome rearrangements that define a "simple" genomic landscape. The main feature of this pattern is gain of chromosome 1p and 16p with loss of 16q [5]. Luminal B subtype has a different pattern of genomic alterations called "amplifier" or "firestorm." This pattern is characterized by focal high-level DNA amplifications, clustered on one or more chromosome arm [6]. Most of luminal B DNA rearrangements affect the expression of genes involved in signaling, cell cycle regulation, and nucleic acid metabolism such as FGFR1, MYC, CCND1, MDM2, ERBB2, and ZNF217. In these tumor cells, the amplified DNA can exist either as repeated units within chromosomes called homogeneously staining regions (HSRs) or as extrachromosomal copies called "double minutes."

Several studies have demonstrated the presence of genomic instability in luminal A/B cancers. Cyclin D1, one of the most amplified genes in luminal B cancer, contributes to tumor progression by activating a transcriptional program that promotes chromosomal instability (CIN) [7]. The analysis of a large dataset of human specimen has shown that 52% of luminal A/B tumors overexpress the gene ESPL1 that encodes for a separase, a protease that cleaves the chromosomal cohesin during mitosis [8]. Indeed, overexpression of ESPL1 in the mouse mammary gland induces chromosomal instability and aneuploidy [9]. Genomic data from over 1000 luminal A tumors has identified four major subtypes defined by distinct copy number and mutation profiles. Among these types, this group has characterized an atypical luminal A subtype characterized by higher genomic instability, TP53 mutations, and increased aurora kinase signaling associated with worse clinical prognosis [5]. Moreover, a recent study has shown that in early-stage luminal breast carcinoma, genomic instability, defined as a high number of chromosomal breakpoints, is a stronger prognostic marker than proliferation [10].

Overall, these studies suggest that genomic complexity is a feature of luminal breast cancer and can be used to predict the outcome of these tumors.

2.3 Genomic Instability in ERBB2 Subtype of Breast Cancers

ERBB2 tumor subtype has a genomic landscape that is similar to luminal B subtype. It is characterized by an "amplifier" genomic pattern with focal high-level DNA amplifications [6]. However, different from luminal B subtype, amplification

of 17q12 (ERBB2) is the most prominent amplification event in ERBB2 subtype. The amplification of ERBB2 gene seems to derive from a sister chromatid breakage-fusion-bridge process based on the analysis of an ERBB2-amplified breast cancer line [11].

Several studies have revealed a direct link between ERBB2 oncogene and factors involved in the maintenance of genomic integrity. For example, the analysis of a collection of ERBB2-positive breast cancer cells suggests the presence of centrosome amplification with increased protein expression of the centrosome kinases Nek2 and Plk4 [12]. Also, expression of ERBB2 in immortalized breast epithelial cells downregulates the DNA damage sensor protein histone H2AX and a number of other components of the HR and NHEJ double-strand DNA break repair pathways [13]. Overall, these preclinical models indicate a role for ERBB2 signaling in initiating CIN and defective cell cycle control.

2.4 Genomic Instability in Basal-Like and Triple Negative Subtypes of Breast Cancers

Basal-like and triple negative (TN) breast cancers have an extremely complex genomic pattern. Their genome includes numerous gains, losses, and small tandem duplications resulting in a highly segmented profile with many copy number transitions. In this landscape a prominent place is occupied by the tumor suppressor BRCA1. The gene BRCA1 is one of the most important DNA repair factors and controls HR-directed DNA repair [14]. Strong similarities exist between breast cancers bearing BRCA1 mutation and sporadic basal-like breast cancers, underlying the inherently genomic instability of this particular cancer subtype.

Germline mutations that inactivate BRCA1 predispose women to breast cancers with basal-like/TN features [15]. In these tumors, dysfunction of HR repair leads to increased error-prone repair, which results in chromosome rearrangements and copy number transitions. Although BRCA1 is mainly known for its role in DNA repair, it has several other biological functions that contribute to tumor predisposition. BRCA1 controls breast epithelial differentiation, regulating the differentiation of ER-negative breast epithelial stem cells into ER-positive luminal progenitors [16]. This study has opened a debate on the cell of origin of BRCA1-associated breast cancers. For example, the breast tissue of women carrying BRCA1 mutations are characterized by abnormal accumulation of luminal progenitors, supporting the idea that basal-like tumors may originate from a cancer-initiating cell with luminal features [17]. Indeed, deletion of BRCA1 in mouse mammary epithelial luminal progenitors produces tumors that phenocopy human BRCA1 breast cancers [18].

Recently, we identified BRCA1 as a novel regulator of cellular antioxidant response [19]. In this study, cells carrying BRCA1 loss-of-function mutations accumulate oxidative stress that affects survival. This is counteracted by the activation of one of the most important pro-survival programs in the cells, the PI3K pathway (see Chap. 1.1 for details), and controlled by estrogen that restores antioxidant defense and promotes survival and malignant transformation [20]. These data clarified the role of estrogen in BRCA1/TNBC as suggested by other mouse and human studies [21, 22].

BRCA1 is not the only DNA repair factor associated with basal-like breast cancers. Other proteins involved in DDR and DNA repair have been identified. These factors are transducers (ATM and ATR) or effectors (Chk2) of DDR and repair factors (Rad51 and PALB2).

Because basal-like and TN breast cancers are associated with a very complex genetic landscape, effective treatment of this particular subtype of breast cancer still represents a challenge for clinicians. Therefore, scientists are devoting their efforts to identify specific vulnerabilities that guide to more targeted therapeutic approaches. For example, basal-like and TN breast cancers seem to rely on the activation of PI3K signaling pathway for cell growth and survival [23]. The loss of PTEN, the negative regulator of PI3K, is a very frequent genetic event in these tumors [24]. Indeed, basal-like and TN breast cancers are particularly sensitive to PI3K inhibitors, such as BKM120 [25]. The activation of PARP-mediated DNA repair (SSB and BER) is another mechanism of adaptation that occurs in BRCA1-mutated cancers that are HR defective [26]. PARP inhibitors have proven to be particularly effective in these tumors, mainly in combination with PI3K inhibitors [27]. Our group has identified another dependency of basal-like and TN breast cancers that involves the activity of the serine/threonine protein kinase PLK4 [28]. This work has led to the identification of CFI-400945, a potent and selective PLK4 inhibitor, particularly effective in tumors with PTEN deficiency. Overall, basal-like and TN breast cancers are characterized by high degree of genomic instability compared to other breast cancer subtypes. Although genomic instability can produce alterations that are beneficial for tumor growth, it can also create vulnerabilities. Importantly, genomic instability can generate "synthetic lethal" interactions that can be exploited therapeutically.

Conclusions

As discussed in this chapter, breast cancer is a very complex disease with different biological and genetic features. The study of breast cancer genomes and genomic instability is advancing rapidly thanks to more advanced genomic technologies, but much remains to be learned. For example, it is unclear which role genomic instability has in the clonal evolution of breast cancer. Genomic instability can be the general driving force of breast cancer or simply a conse-

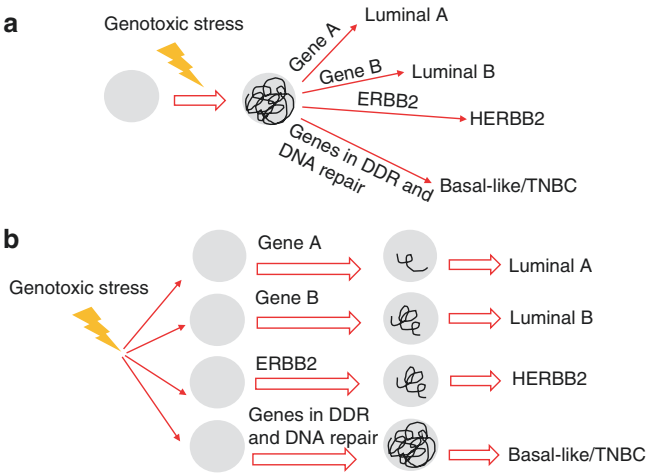

Fig. 2.2 Hypothetical model of the evolution of breast cancer in relationship with genomic instability. (**a**) All breast cancer subtypes originate from a common cancer-initiating cell. Upon endogenous or exogenous genotoxic insult, a genome becomes unstable and stochastically generates the mutational event/s that drive/s the development of each subtype. (**b**) Each cancer subtype derives from a specific cell of origin. In condition of genotoxic stress, each cell type acquires specific genetic mutations that drive different subtypes. Accordingly to which pathway is affected by the mutation, each tumor type will acquire different degrees of genomic instability

quence of the specific mutations that characterize each subtype (Fig. 2.2). In the first hypothesis, all subtype would share a common cell of origin that acquires a certain level of genomic instability upon genotoxic damage. This mutator landscape would favor stochastic acquisitions of mutations in specific genes such as ERBB2 that would give rise to each subtype. In the second hypothesis, each subtype would originate from a different cell that will stochastically acquire mutations in specific genes such as ERBB2. Based on which pathway will be altered, each subtype will show a different degree of genomic instability. Both hypotheses well support the heterogeneity of breast cancer disease. The recent development of single-cell sequencing may reveal another layer of complexity, that is, intra-tumoral heterogeneity. This analysis can be used to identify and characterize hidden subpopulations and shed light on the clonal evolution of breast cancer. Clonal dynamics should be studied in response to cancer therapy to further evaluate mechanisms of adaptations. Currently, computational biologists and bioinformatics have developed tools that are able to handle huge amount of data. The task for cancer research scientists is to develop biological models suitable to reveal "drivers" and "vulnerabilities" in the overwhelming landscape of cancer complexity. Although advantageous, living with genomic instability is a challenge that cancer cells face by triggering mechanisms of adaptation. The identification of these mechanisms will reveal novel vulnerabilities for better-tailored therapies.

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Fundamental Pathways in Breast Cancer 3: Estrogen Biology

3

Luca Magnani and Darren K. Patten

3.1 Introduction

Over the last two decades, it has become evident that breast cancer should be considered as a family of diseases rather than as a unique malignancy. Pathological, molecular, and genetic analysis have revealed the existence of five to ten main subgroups [1–3]. Over 70% of all patients are generally classified by the tumor dependencies on estrogenic compounds [4]. These dependencies are principally mediated by the nuclear receptor estrogen receptor α (ERα) [5, 6]. For all these reasons, ERα remains the key driver in the majority of breast cancers and is commonly used as a molecular biomarker for stratification while serving as the main target for systemic adjuvant chemotherapy. In this chapter we will discuss the molecular mechanisms of ERα activation, focusing on integrative analysis that have recently exposed the intimate link between ERα and chromatin structure.

3.2 Estrogen Signaling and the ERα Underlie a Large Fraction of Breast Cancer Hallmarks

A critical shift in the approaches to studying estrogen biology has occurred in the last decade. The field has gradually moved from the investigation of single genes to the study of model cell lines and finally moving to patient-derived samples. This broadening involved also the molecular toolkit used by scientists through the development of next-generation sequencing and allowed the development of unbiased, genome-wide assays [7]. This transition was critical to refine our understanding and overtake long-standing dogmas. Since then, the field has become aware of the complexities of estrogen signaling and began examining the association between DNA, the scaffolding DNA structure (chromatin), epigenetic and genetic factors, and ERα. Using system biology approaches and genome-wide annotations, we now have also linked ERα to the majority of breast cancer hallmarks thus reemphasizing the importance of this dogmatic transcription factor.

The involvement of estrogen signaling in breast cancer biology was recognized over a century ago, when a causative link between ovariectomy and breast cancer progression was made [4]. Several studies have also linked estrogen and breast cancer etiology. Some of the best-characterized predisposing factors predisposing factors leading to breast cancer breast cancer reflect endogenous estrogenic exposure (reviewed in [8]). In addition, additional exogenous estrogen exposure can also favor the development of luminal breast cancer [9]. Over 5×10^3 studies (source, PubMed) have evaluated the role of estrogen signaling in MCF7 cells, one of the preferred tools to investigate the dynamics of estrogen signaling at a molecular levels. The most investigated aspect of estrogen signaling is without any doubt the sustained growth promoted by activated ERα. Nonetheless, estrogen signaling is also involved in many other cancer hallmarks [10] (Fig. 3.1). Some of the molecular details of how this happens will be discussed in more detail in other sections of this chapter and other chapters as well.

Estrogen signaling has been extensively associated with evasion of cell death [11], invasion and metastasis [12, 13], and inflammation [14] phenotypes. More recently estrogens and ERα have also been associated with angiogenesis [15], genome instability and mutations [16], and deregulation of cellular energetics [17, 18]. It is important to understand that most of these biological features are modulated by activated ERα at the DNA level. In the next sections, we will discuss how breast cancer cells can access such a wide array of cellular response via a single transcription factor.

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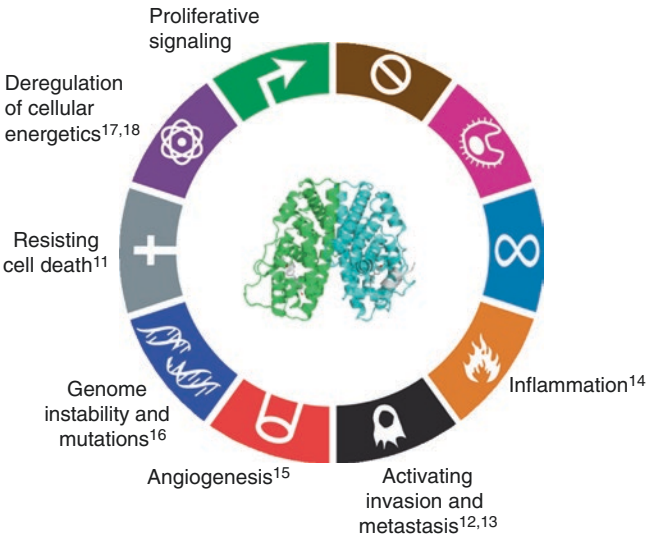

Fig. 3.1 ERα directly controls the majority of cancer hallmarks in breast cancer cells. The image is modified from [10]

3.3 This Must Be the Place: A Brief Introduction of the Chromatin Environment

ERα signaling can be broadly classified into canonic (genomic) and noncanonical (non-genomic). Noncanonical signaling involves ERα activation in the cytoplasm and the subsequent activation of complex signal transduction cascades mediated mostly by kinases. For an in-depth review of the subject, see [19]. An example is provided by EGF-EGFR signaling converging on ERα phosphorylation. Even in these scenarios, ERα ultimately acts via DNA binding [20, 21]. A more controversial line of investigation has addressed the potential role of ERα in the cell membrane [22], the data though have been challenged [23] and the field has not matured a consensus. On the other hand, a large fraction of ERα molecules constantly shuffle between the cytoplasm and the nucleus where they alternate between a free-floating state and a DNA bound state [24, 25]. More importantly, canonic ERα signaling has been associated with the majority of the breast cancer hallmarks discussed above. For all these reasons, we will focus the discussion on canonical signaling.

The full-length ERα contains a ligand binding domain (LBD) and a DNA binding domain (DBD) separated by a hinge domain [26]. Once the ligands contact the LBD, conformational changes occur throughout the entire protein and allow for dimerization and DNA binding [27, 28]. ERα then quickly contacts the DNA at genetically defined DNA sequences called estrogen-responsive elements (EREs) [29]. More than 70,000 EREs are scattered throughout the human genome in addition to regions that harbor half or degenerate EREs which are also permissive to ESR1 recruitment [30]. This poses the question of how many regions ERα binds throughout the genome, how ERα finds these ERE, what are the molecular determinants of ERα binding, and how many ERα are actually functional.

The human genome consists of around 3×10^9 base pairs. Eukaryotes have evolved strategies to compact this vast array of information in the nucleus via higher-ordered packaging (the chromatin). 147–148 bp of the DNA string wrapped around histone proteins is the minimal repeating unit of the chromatin (the nucleosome) [31]. ERα, similarly to 94% of all DNA binding proteins, has higher affinity for nucleosome-free DNA [32, 33]. Thus, chromatin accessibility represents the first barrier to ERα binding. ERα binding is the primary driver of gene expression. Activation of ERα induces the strong transcriptional response that drives breast cancer cell proliferation [34]. ERα orchestrates transcription by binding at critical DNA regions known as regulatory elements [35]. These regions can be broadly classified as promoters and enhancers based on the relative distance from the gene that is controlled (Fig. 3.2). The chromatin environment at regulatory regions is defined by several well-characterized epigenetic features [36]. For example, active promoters and enhancers are typically nucleosome-free and accessible to transcription factors [33]. The nucleosomes surrounding regulatory regions carry special chemical modifications on the histone tails depending on their activity status [36–38]. These modifications, known as histone post-translational modifications (HPMTs), have been extensively used to annotate regulatory regions in the genome by several international consortiums [36, 39, 40]. Promoters are characterized by histone 3 lysine 4 tri-methylation (H3K4me3), while enhancers are generally enriched for histone 3 lysine 4 mono-methylation (H3K4me1) [38]. On the other end of the chromatin spectrum, inactive/repressed regulatory regions carry H3K27me3 and H3K9me3 modifications [41]. Collectively, chromatin accessibility and histone modifications are a constitutive part of the epigenome. Several integrative studies have now dissected the relationship between ERα and the epigenome.

Fine mapping of ERα DNA interaction and integration with epigenetic data were central to remodel a long-standing dogma in ERα biology. For a long time, it was hypothesized that ERα controls transcription by binding to primarily the promoters of target genes. It is now well established that 97% of ERα binding occurs at distal enhancers [35, 42, 43] (Fig. 3.1). These regions are typically enriched for active epigenetic modifications (H3K4me1, H3K27ac) and devoid of repressive marks (H3K27me3 and H3K27me9). These unex-

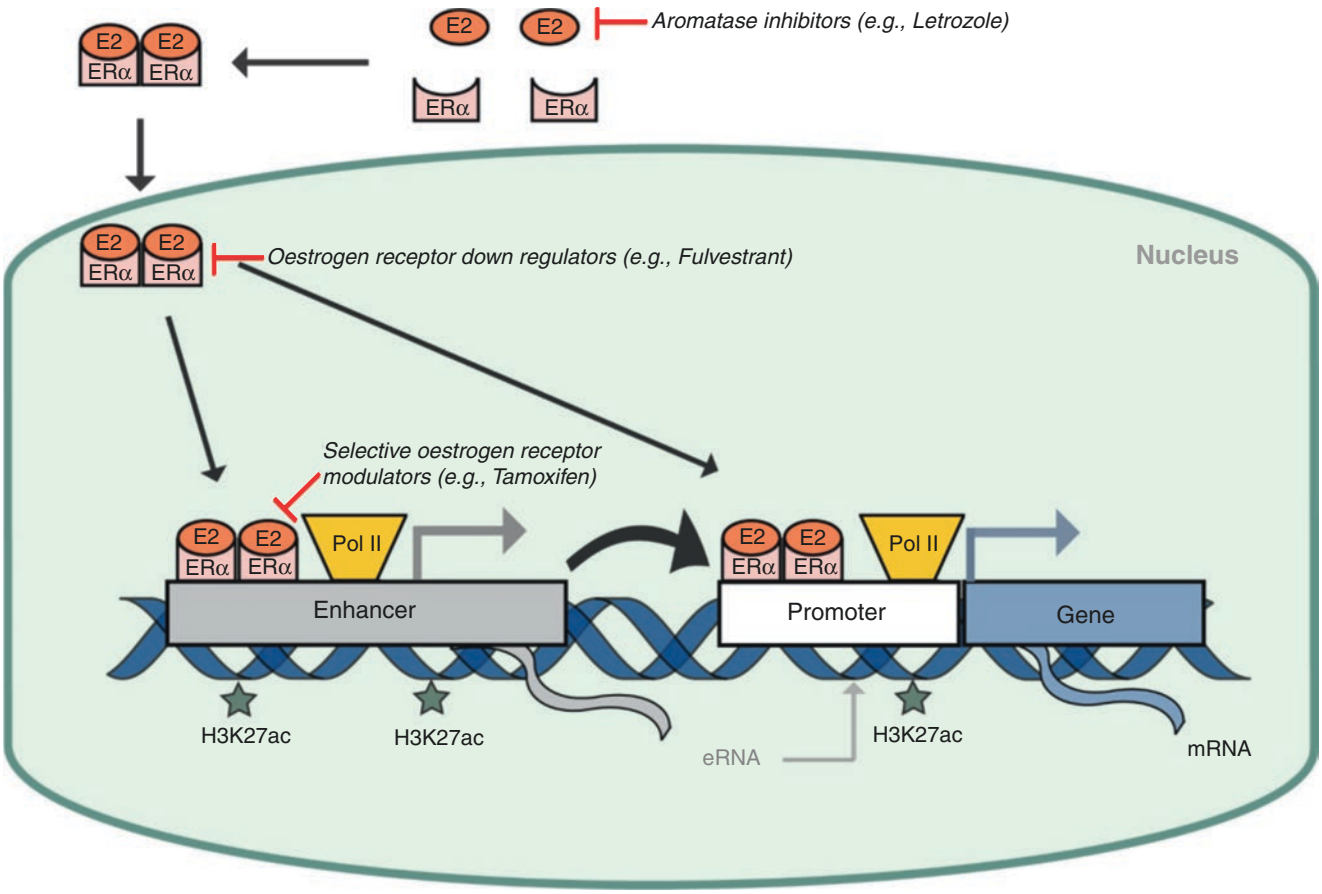

Fig. 3.2 ERα interacts with the chromatin to regulate gene expression. ERα is found both in the cytoplasm and in the nucleus prior to estrogen-mediated activation. Estrogen activation is the main target of endocrine therapies. Once activated, the receptor binding regulatory regions (enhancers and promoters) that contain ERE motif are bookmarked by specific histone modifications. ERα binding potentiates gene transcription by Pol II and leads to the activation of many genes involved in proliferation, invasion, and other cancer hallmarks

pected landmark discoveries have provided the foundations for the last ten years of research looking for the modalities by which ERα mechanistically modulate gene expression.

3.4 Pioneer Factors Are Critical Regulators of ERα Binding to the Chromatin

ERα can bind the DNA only when activated; otherwise, it remains unbound within the cytoplasm/nucleus. Several groups have examined the dynamic properties of ERα binding to the DNA. Biochemical investigation using the average signal from millions of cells have established a paradigm whereby once activated, ERα cyclically binds to target DNA at 45-min intervals [35, 44–46]. Nonetheless, ERα requires nucleosome-free regions for efficient DNA binding. Recent studies have shown that ERα binding sites are maintained in an open chromatin conformation by a specialized set of transcription factors called pioneer factors [47, 48]. While ERα interaction with the DNA is ligand and time dependent, pioneer factors bind near EREs in the absence of external stimuli and are thought to maintain more stable interactions with the chromatin. Two of the best-characterized pioneer factors are FOXA1 [35] and PBX1 [24]. Depletion of FOXA1 and PBX1 results in a dramatic reduction in chromatin accessibility at local EREs [24]. In contrast with other transcription factors, pioneer factor can interact with nucleosomes and bind nucleosome-dense DNA [49] thus increasing chromatin accessibility *de novo* [50]. Pioneer factors also appear to be the link between the epigenome and ERα via nucleosome modifications. Several evidences indicate that pioneer factors might be able to interact with nucleosome modifications. For example, overexpression of a protein involved in erasing the H3K4me2 mark corresponds to a loss of PBX1 and ERα from several enhancers [24]. Furthermore, pioneer factors are found mainly at H3K4me1/2 rich regions [24, 51]. In summary, pioneer factors act as the gatekeepers of potential ERα binding sites by modeling chromatin accessibility and bookmarking a discrete number of genomic locations for ERα (Fig. 3.3).

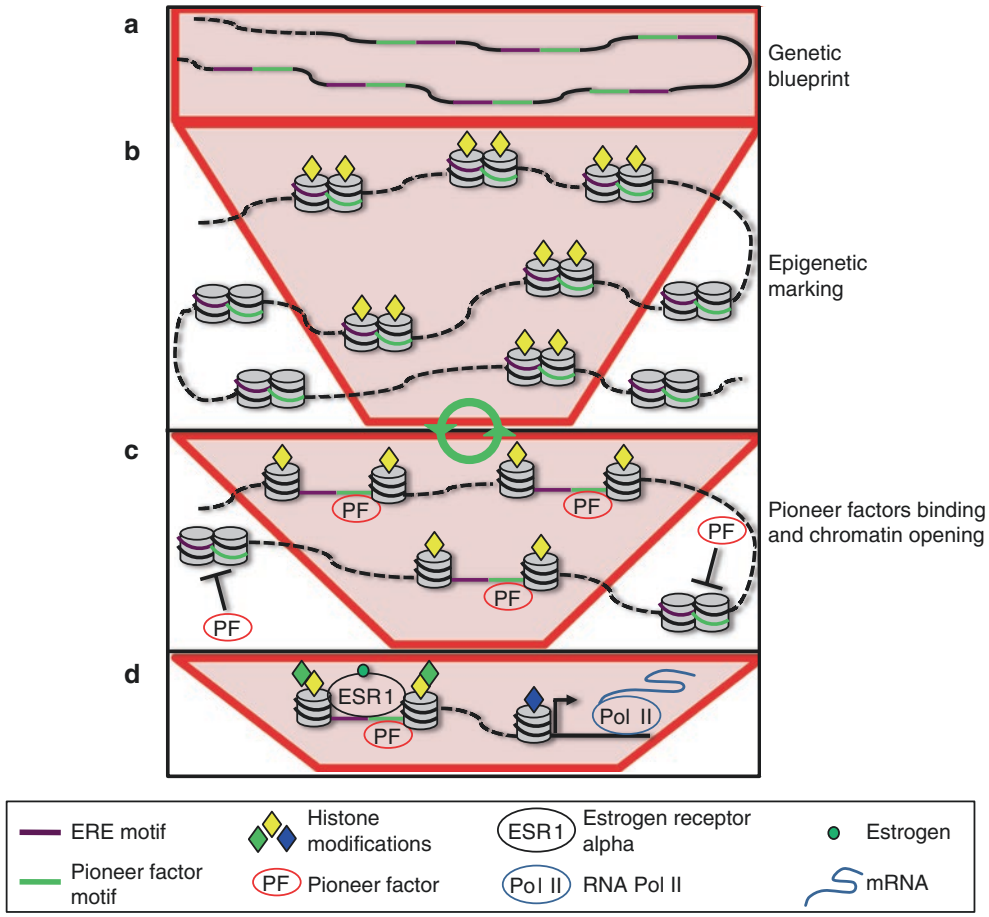

Fig. 3.3 (**a**) The chromatin template mediates ESR1 signaling activity. ERE elements are distributed across the genome and can co-localize near pioneer factor motif elements. (**b** and **c**) Histone modifications (e.g., H3K4me1/2) and pioneer factors cooperate to increase chromatin accessibility at a subset of genomic ERE. (**d**) These events promote competence for successful ESR1 binding in response to external stimuli and mediate ESR1 transcriptional program (reproduced with permission from [6])

3.5 ERα Regulates Transcription via Chromatin Looping

The vast majority of ERα binding occurs at distal regulatory regions (enhancers). How does ERα then activate gene transcription? It is well established that activated ERα is essential to promote efficient RNA polymerase II release from gene promoters and enhancers as well [52–54]. Recent advances in chromatin conformation capture assays have highlighted the tremendous complexity of the 3D organization of the chromatin [55, 56]. These genomic assays characterized thousands of enhancer-promoter interactions partially explaining how distal regulatory regions can mediate transcription. Not surprisingly, ERα was one of the first transcription factors found at interacting chromatin loops [57]. There are, however, some unresolved questions about the formation of these loops. One model postulates that estrogen-activated ERα can drive loop formation [57–59]. However, there is also an indication that ERα might exploit preformed loops that have been set up by pioneer factors with the contribution of epigenetic modifications (reviewed in [60]). It is conceivable that future studies will find that ERα chromatin looping is very context dependent and could include both models of transcriptional activation.

3.6 ERα Regulates Transcription via Protein Recruitment

ERα regulates transcription by modulating RNA polymerase II release from the 5′-prime end of the gene body. Yet, ERα alone is not sufficient for full transcriptional activation. It soon became apparent that ERα recruits several other proteins to promote transcription [46, 61]. These studies also explained how ERα could modulate repression. The proteins recruited by ERα are commonly referred to as coactivators or corepressors. Interestingly, two among the first coactivators to be identified (SRC1 and BRG1) are critical chromatin modulators, further highlighting the strong link between ERα and the chromatin environment. Steroid receptor coactivator-1 (SRC1) is a histone acetyltransferase [62] (Histone 3 lysine 9/14 acetylation), while BRG1 is a chromatin remodeler [63, 64]. Histone 3 lysine 9 acetylation is yet another HPMTs strongly associated with active transcription and has been shown to be important for chromatin relaxation and improved DNA accessibility [65]. On the other hand, histone acetylation provides a docking station for bromodomain proteins, including the chromatin remodeler BRG1. These proteins interact with acetylated histones and are essential to remodel and reposition nucleosomes [66]. These

cofactors mostly lack DNA binding abilities and rely on ERα for recruitment at the correct regulatory regions. Several lines of evidence support this model. For example, it has been noted that BRG1, SRC1, and other cofactors parallel ERα cyclical pattern of recruitment onto the DNA [44]. More importantly, blocking ERα binding is sufficient to abrogate binding for several cofactors [67]. The list of cofactors has been growing dramatically in the last few years. Proteomic-based approach has now identified hundreds of potential coactivators and corepressors [68] including several ERα target genes themselves. Collectively, these examples emphasize the complex transcriptional machinery driving growth in breast cancer cells while underscoring the central role of ERα in coordinating all genomic actions.

3.7 Alternative ERα Binding Programs Correlate with Differential Patient Outcome

ERα binding is modulated by chromatin accessibility, epigenetic modifications, and cofactor recruitment. The combination of these regulatory layers shapes cell type-specific ERα binding. But are alternative ERα binding combinations reflective of different biology? Could alternative ERα binding be used to stratify breast cancer patients *in vivo*? A recent study from the Carroll group have examined, for the first time, the collection of ERα binding (known as cistrome) in several luminal breast cancer patients characterized by distinct outcome [43]. The data suggest that while a lot of ERα binding seems to be patient-specific, there are also clusters unique to good outcome patients and clusters unique to poor outcome patients in addition to a core ERα cistrome common to patients and cell lines as well [43]. Of note, differential ERα binding is potentially correlated with alternative transcriptional programs. Gene expression profiling using putative ERα target genes can also identify subgroups of patients with dramatically different outcome suggesting that ERα can guide both aggressive and nonaggressive breast cancers. An explanation for these patterns can be found in alternative usage of pioneer factors. For example, it was shown that when ERα interacts with PBX1, it can guide transcription of genes associated with aggressive phenotype [24]. On the other hand, ERα interaction with GATA3 [47, 69, 70], another breast cancer pioneer factor, seems to be associated with less aggressive tumors ([43]).

Genetic alteration can also impact ERα recruitment in vivo. Genomic analyses have revealed that about 20% of all luminal breast cancer patients have copy number loss at the progesterone receptor (PGR) locus [71]. PGR is one of the best-characterized ERα target genes and is commonly used to stratify luminal breast cancer patients into luminal A (ER+/PGR+) and luminal B (normally ER+/PGR− or ER+/PR+ and HER2+) subtypes [72]. Nevertheless, PGR has been also described as an ERα cofactor capable of hijacking ERα upon native progesterone stimulations [71]. More importantly, the ERα cistrome obtained from progesterone-treated cells correlate with milder phenotypes and improved outcome. Indeed, patients with PGR copy number loss are characterized by a poorer outcome [71]. In summary, ERα genomic localization has significant effects on tumor biology. These data can be then harnessed clinically by finding practical strategies to reprogram ERα. For example, it has been postulated that native progesterone (but not synthetic progestin) treatment in PGR wild-type patients might carry significant benefits.

3.8 Alternative Means of ERα Activation

Estrogen signaling plays an essential role in driving breast cancer growth at early stages. All approved adjuvant systemic therapies are in fact designed to block estrogen signaling (for an updated review see [73]) (Fig. 3.2). Targeting estrogen signaling lowers the rate of relapse by about 50% in ERα-positive patients [74]. However, it is becoming apparent that estrogen signaling remains central at later stages of the disease as well. In the last three years, it has been shown how ERα-positive breast cancer cells develop alternative strategies to activate the receptor in later stages of the disease. There are two main mechanisms through which this can happen. The first involves activating mutations targeting the LBD [75, 76]. Two independent studies found that metastatic breast cancer patients with a history of luminal disease have a significant prevalence (~20%) of mutations targeting the LBD of ERα. These mutations appear to activate the receptor in the absence of estrogens through conformational changes. This results in a constitutively active form of the receptor that cannot be turned off by conventional chemotherapy. It remains unclear at what stage of the disease these mutations arise, since the patients in which they were identified received an extensive array of treatments [75, 76].

The second mechanism involves the activation of cholesterol biosynthesis in estrogen-independent ERα breast cancer (i.e., letrozole resistant) [18]. In this case, ERα cancer cells develop the ability to synthesize *de novo* an alternative ERα ligand (27-hydroxycholesterol) [77]. This in consequence allows estrogen-independent, ERα-dependent proliferation [18]. Moreover, 27-hydroxycholesterol was previously shown to stimulate an invasive phenotype in ERα breast cancer mouse models [12]. Ultimately, estrogen signaling might become redundant as the disease approaches the later stages despite breast cancer cells remaining frequently ERα positive [78]. This is also reflected clinically by the limited benefit of ERα downregulators such as Faslodex [79]. In summary, estrogen signaling and ERα continue to play a key role throughout the patients' entire journey.

3.9 Novel Insight in the Genomic Activity of ERα

Estrogen signaling is essential to promote growth, invasion, and survival of breast cancer cells. In addition, recent studies have also linked ERα and estrogen signaling to genetic instability and mutational burden. One of the most recently identified ERα cofactors is the cytosine deaminase APOBEC3B [16]. APOBEC3B was previously linked to a specific mutational signature (C to T) in breast cancer patients [80, 81]. Interestingly, APOBEC3B is temporarily co-recruited on the chromatin along with ERα and depletion of ERα results in loss of APOBEC3B recruitment [16]. One of the key findings however is that APOBEC3B is essential for ERα transcriptional activity. Moreover, estrogen stimulation in ERα-positive cell lines was sufficient to jump-start DNA repair mechanisms and the accumulation of double-strand breaks at ERα binding sites [16]. Why estrogen activity induces risky double-strand breaks? Mechanistically, these findings fit with the idea of chromatin remodeling at ERα regulatory regions. While the transcriptional machinery advances, it might require relax and unwounded DNA [82, 83]. Nonetheless, cells with inefficient DNA repair might then have an increased mutational burden at regulatory elements. Altogether these data suggest that estrogen signaling and ERα might also contribute to the mutational signature found in ERα breast cancer patients.

Conclusions

In this chapter, we have discussed some of the critical roles of estrogen signaling in breast cancer cells. By using integrative analysis, we are finally addressing the question we are finally addressing the question as to why ERα is so dominant in breast cancer cells. Yet, some aspects remain uncertain. For example, is ERα binding important in the context of breast cancer predisposition? A recent study found that single nucleotide polymorphisms (SNPs) associated with increased breast cancer risk have a significant tendency toward EREs and FOXA1 binding sites [84]. Possibly, these SNPs act by modulating ERα and other pioneer factors binding to DNA [85]. It is fascinating how then the ERα might evolve during the patient journey. If ERα is involved in increasing the mutational burden, it is then easy to speculate that some of these mutations might increase affinity for ERα, while others might decrease it. Consequently, the ERα cistrome might change at high frequency allowing the tumor to transform during progression and activate or adapt many of the cancer hallmarks in response to change in tissue, therapy, and many other physiological parameters.

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Fundamental Pathways in Breast Cancer 4: Signaling to Chromatin in Breast Development

4

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4.1 Breast Development and Its Relationship with Cancer

4.1.1 Normal Breast Development and the Identification of Mammary Stem Cells

Development of the breast has important differences compared to other organs, as it is mostly completed in the postnatal life (for extensive review of breast development, see [1]). At birth, the breast consists only of a rudimentary ductal structure populating the area around the nipple. During puberty and in response to ovarian hormones, a branched ductal architecture develops, driven by highly proliferative "terminal end bud" (TEB) cells. This process is macroscopically and molecularly similar to the epithelial-to-mesenchymal transition (EMT) that occurs earlier in embryonal development and implies infiltration of epithelial cells into the underlying fibroadipose stroma. Postpubertal mammary ducts so formed consist of a bilayer of polarized **luminal** cells surrounded by myoepithelial **basal** cells; small milk-producing alveoli with apocrine **lobular** cells develop; lastly, a specialized stroma with trophic function surrounds the mature gland. Ductal and alveolar cells undergo periodic waves of apoptosis and regeneration at each estrous cycle. During pregnancy and then lactation, duct arborization and alveolar volume increase dramatically through active cell proliferation.

The cell types and molecular mechanisms governing breast regeneration are still incompletely understood. Early experiments showed that any portion of the mammary gland transplanted into a suitable environment (cleared mammary fat pad for mouse-to-mouse transplants, kidney capsule for human-to-mouse) is able to regenerate a functional mammary gland [2, 3]. However, it was unclear whether this ability is shared by most cells or is restricted to few mammary stem cells (MaSCs). The latter view is now supported by a large body of experimental evidence (reviewed in [1, 4, 5]), but the heterogeneity of the experimental systems employed has fueled debate about the features of MaSCs. Earlier transplantation-based experiments revealed that single individual cells able to completely regenerate a functional gland and give rise to all mammary lineages can be prospectively identified in the mouse as $CD24^{+}$ or $CD29^{hi}/CD49f^{hi}$ [6–8]. In humans, $CD49^{+}/EpCAM^{low}$ cells or aldehyde dehydrogenase (ALDH)-active cells are enriched for regenerative activity when transplanted in immunocompromised mice [9, 10]. Furthermore, in vitro mammosphere formation assays revealed that regenerative activity is highly enriched in a rare population that cycles infrequently [11, 12]. This led to a dominant model in which mammary regeneration is carried out by few and slow-cycling stem cells with the ability to give rise to all mature breast lineages, in clear resemblance to models of hematopoiesis. However, transplantation is a highly nonphysiological setting in which the revealed developmental potential may not reflect the actual contribution to normal mammary homeostasis. More recent in vivo lineage tracing experiments have established the existence of cells with "true" multipotency but also revealed that multiple reporter alleles can identify multipotent cells with different expression profiles, frequency, and cell cycle dynamics, suggesting a higher than expected heterogeneity [13–15]. A significant part of adult mammary lineages is replenished by self-renewing "progenitor" cells whose contribution is restricted to the luminal or basal lineages [16, 17]. A subset of self-renewing cells is retained through rounds of pregnancy-induced alveolar remodeling and re-initiates alveologenesis at subsequent pregnancies in mice ("parity-induced mammary epithelial cells," PI-MEC) [18–21], although the existence of such cells has never been demonstrated in humans [4]. It is still unclear whether multipotency is a fixed feature of cells which are multipotent by transplantation *and* by lineage tracing, or whether unipotent

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progenitors might become multipotent upon strong changes in environmental signals, like physiological hormonal changes or transplantation.

4.1.2 Relationship Between Normal Breast Development and Breast Cancer

The study of normal developmental dynamics can provide important information to understand breast cancer natural history. What is the cell of origin of breast cancer? Multiple cumulative genetic abnormalities are required to transform a normal cell. In the breast, on average one mutation per coding megabase are identified in large-scale sequencing projects [22, 23]. As the rate of mutation accumulation at each cell division is low [24], cells of cancer origin are likely to have a long proliferation history: MaSCs or progenitors surviving multiple cycles of gland regeneration are the likeliest candidates.

But does this also imply that the developmental potential of the cell of origin can shape cancer phenotype? This was strongly suggested by the landmark expression microarray studies of the early 2000s [25, 26], which showed that groups of commonly expressed genes that define normal lineages are also able to define clusters of cancers with common natural history ("intrinsic" subtypes). However transcriptional similarities do not necessarily extend to the cell of origin, implying for instance that a basal-like cancer had to originate from a basal-restricted cell. In fact, more recent studies on BRCA-deficient mice and humans suggest that basal-like cancers derive from luminal-committed progenitors [9, 27]. The emerging consensus is that luminal progenitors are the likely cell of origin for both luminal and basal-like tumors, whereas MaSCs and/or basal progenitors are the initiators of the rarer claudin-low subtype. The origin of HER2+ tumors is less well understood; data in mice suggest that parity-induced stem cells might be the culprit as their ablation inhibits tumorigenesis in MMTV-neu mice [28], but to what extent this model really mimics human HER2+ tumors is disputable since the murine expression profile is more akin to human luminal tumors [4, 28, 29]. Almost completely undetermined is the cell of origin of lobular and rarer breast cancer subtypes. Transcriptome analysis of "special" subtypes (like mucinous and micropapillary) all clustered in a separate group, revealing transcriptional homogeneity despite morphologic differences, but little about a possible common cell of origin [30]. Lobular cancers showed heterogeneous signatures resembling ductal subtypes (including luminal-like and basal-like signatures) [30]; transcriptome signatures within lobular cancers have been recently studied in more depth by the TCGA, with the definition of three new subgroups "reactive-like," "immune-related," and "proliferative" [31]. Again this reveals little about the cell of origin, but the only available genetically engineered mouse model showed that lobular tumors can be obtained from basal cells expressing cytokeratin 14 [32].

4.2 Signaling Pathways Controlling Breast Development and Their Alterations in Cancer

Several authors have proposed that molecular mechanisms specific for stem cells might also be responsible for malignant behavior, in a declination of the "cancer stem cell" theory [33]. Specifically, the same pathways allowing stem cells to maintain multilineage potential resist to physiological death and invade the stroma to (re)generate the organ, might also allow cancer cells to adapt their transcriptome, resist to treatment-induced death, and become invasive and metastatic. We will now review the most important developmental and stem cell-related pathways in breast. Then, we will review how these signals converge on chromatin (the ensemble of normal DNA and DNA-bound proteins) to modulate transcription. Chromatin can directly (by physical compaction) or indirectly (through differential recruitment of effector proteins) control DNA accessibility to transcription factors and stabilize phenotypic traits by restricting the degree to which transcriptional signatures can be further modified by competing signals. Hence, proteins directly governing chromatin architecture are particularly important in developmental processes and are often disrupted in cancer.

4.2.1 NOTCH/NUMB and p53 as Regulators of Symmetric vs Asymmetric Cell Division

4.2.1.1 NOTCH Pathway in Breast Development

The NOTCH pathway governs cell lineage determination and body patterning in all metazoans [34–36]. NOTCH was classically identified as a regulator of neuroectodermal development in *Drosophila* but then emerged as a functional module repeatedly exploited in heterogeneous developmental contexts to execute binary cell fate choices, generating and maintaining phenotypic "boundaries" within organs. The four NOTCH receptors are functionally redundant [37] transmembrane proteins with homology to eGFR; once activated by short-range ligand binding, usually requiring cell-to-cell interaction, the receptor is cleaved by the gamma-secretase complex and transported into the nucleus. Here, it induces the assembly of a highly conserved protein complex that canonically includes CBF-1 and RBP-J, plus additional chromatin-modifying enzymes [38]; this drives transcription of NOTCH target genes, most notably the HES and HERP families of bHLH transcription factors [39, 40].

The half-life of the activated receptor is normally very short due to efficient proteasomal degradation, which is dependent on the C-terminal PEST (rich in proline (P), glutamic acid (E), serine (S), and threonine (T)) domain common to all NOTCH receptors. The NOTCH molecular circuitry is reinforced by multiple layers of intrinsic and extrinsic feedback mechanisms ("lateral inhibition") that amplify small variations in ligand/receptor concentrations between adjacent cells; this allows the emergence of discontinuities among cell populations with prior equal potentials. A particularly important regulatory role is played by NUMB, a membrane-associated protein that targets NOTCH receptors to proteasomal degradation and is a key determinant of asymmetric cell division: in several NOTCH-dependent lineage choices, NUMB is unequally partitioned between daughter cells, leading to differential inactivation of the NOTCH pathway. Numb also independently regulates the stability of p53 [41], which in itself is implicated in stem cell self-renewal and (a) symmetric cell division [11].

These general functional properties also apply to NOTCH role in breast biology. NOTCH pathway receptors are expressed in the luminal compartment [42–44], and its target genes are upregulated in luminal progenitors in the adult gland [42]. This expression pattern is mutually exclusive with that of ΔNp63, which promotes and maintains basal cell gene expression; in fact, NOTCH and p63 appear to be functional antagonists [45]. Hyperactivation of NOTCH pathway by overexpression of the active form [42] or conditional ablation of NUMB [44] leads to ductal hyperplasia with luminal differentiation. On the contrary, ablation of NOTCH function by conditional deletion of the common transcriptional mediators CBF1 or RBP-J led to expansion of the basal cell pool during pregnancy [46]. Collectively, these results suggest a model in which NOTCH pathway activation promotes the commitment of dividing stem cells and progenitors to the luminal lineage at the expenses of the basal/myoepithelial lineage [47]. However, this model is complicated by the presence of low levels of NUMB and NOTCH receptors also in basal and other cell types [42, 44]; the precise mechanism of action of NOTCH in normal mammary gland biology remains an active field of research.

4.2.1.2 Modes of NOTCH Pathway Alterations in Breast Cancer

Although altered NOTCH receptors have been found to act as tumor suppressors in some circumstances [48, 49], in breast cancer and in most other tumors (most notably T-ALL), they behave as classical proto-oncogenes that become constitutively activated through loss of extrinsic or intrinsic regulation [49, 50].

Loss of extrinsic regulation is achieved by genetic ablation of the N-terminal extracellular domain. Early on in the history of breast cancer experimental research, this mechanism was identified as a consequence of insertional mutagenesis of the mouse mammary tumor virus; breast-specific expression of the truncated form induces expansion of luminal progenitors and mammary tumors in experimental animals [51–53].

Among human tumors, alterations in NOTCH receptors are present in around 5% of all cases in different patient populations. Unlike MMTV insertions in the mouse, the generation of an extracellular domain-defective protein is uncommon in humans; this was observed as the result of chromosomal translocations in a recent study [54, 55]. More common are point mutations that frequently (around 60% in the TCGA cohort) are truncating and clustered at 3′ exons, resulting in a disrupted PEST domain and predicted to lead to increased protein half-life. The PEST can also be lost through deletions or translocations [54, 55]. The remaining point mutations are scattered throughout the gene body with no detectable pattern but tend to occur in highly conserved residues important for receptor heterodimerization associated with increased activity in T-ALL [54]. NUMB is frequently downregulated at the protein level in breast cancer, although the mechanism leading to downregulation has not been extensively studied. Deletions can be observed in 0.6% of all TCGA breast cancer patients.

NOTCH/NUMB alterations are strongly associated with HER2/ER/PgR negativity [41, 54, 56] and, as expected, with unfavorable outcome in invasive carcinoma [41, 43, 56–59] and with higher recurrence rate in DCIS [60]. This makes NOTCH pathway an attractive target for drug development. Inhibiting NOTCH through genetic [61, 62] or pharmacological [60, 63] means results in a loss of in vitro self-renewal ability in mammosphere assays.

NOTCH inhibitors are currently undergoing early phase clinical evaluation in breast cancer and other tumors. Two main approaches are being explored: the use of antibodies that disrupt ligand-receptor interaction and inhibitors of the gamma-secretase activity first explored in Alzheimer's disease [64–66].

4.2.2 The Wingless (WNT) Pathway

4.2.2.1 Wnt Pathway in Breast Development

The Wingless (WNT) pathway plays a crucial role in mammary development; similarly to NOTCH, it acts prevalently at short range as a functional module that is repeatedly used in highly different contexts to give rise to variable outputs, including the regulation of asymmetric cell division. Signals are instructed through paracrine cellular communication between the lipidated Wnt ligands and the Frizzled transmembrane receptors. This results in the phosphorylation of the canonical WNT mediator beta-catenin by casein kinase I (CKI) and glycogen synthase-3β (GSK-3β), resulting in its stabilization and nuclear translocation. In the nucleus,

beta-catenin activates transcription of conserved targets, namely, telomerase, Axin2, and LGR5, through TCF/LEF factors. Signal strength is intrinsically regulated through proteasomal degradation of Frizzled receptors by Rnf43/Znrf3, which is in turn inhibited by LGR5; LGR5 marks WNT-responsive cells in a variety of epithelial tissues [67], and in the breast it marks a subset of bipotential stem cells able to give rise to luminal and myoepithelial cells as defined by lineage tracing experiments [13]. PROCR is another WNT target that also marks multipotent mammary stem cells, although intriguingly, PROCR$^+$ cells appear distinct from LGR5$^+$ [14].

Another level of WNT modulation is through sequestration of beta-catenin to adherent junctions by E-cadherin: this peripheral pool of beta-catenin is unavailable for nuclear translocation and is thought to play a role in epithelial-to-mesenchymal transitions [68, 69].

4.2.2.2 Modes of WNT Pathway Alterations in Breast Cancer

Similarly to NOTCH, the Wnt1 receptor was found early on as a common MMTV integration site [70], and the oncogenic potential of Wnt hyperactivation subsequently demonstrated by MMTV-induced overexpression of several Wnt receptors or of beta-catenin [71–75]. This leads to anticipated lobuloalveolar overgrowth, morphologically similar to that induced by pregnancy but with an expansion of poorly differentiated cells [73]; importantly, this is also true in male mice and upon transplantation into ovariectomized recipients, suggesting that Wnt pathway lies downstream of ovarian hormones and that Wnt-aberrant cells might become estrogen independent [76, 77]. As mice age, invasive ductal tumors develop with a penetrance of 100% by 1 year. The long penetrance suggests that additional mutations are required to achieve the invasive phenotype, but importantly ablation of Wnt signaling is still required after the invasive tumor has formed, although loss of p53 facilitates the transition to WNT independence [78].

WNT-hyperactivated mouse models have been used extensively in basic research, but their relevance to clinical practice might be questionable, since components of the canonical Wnt pathway are not frequently mutated in breast cancer [76, 79]. However, aberrant beta-catenin staining patterns (i.e., prevalence of nuclear pattern) is observed in about 20% of ductal carcinomas and, as it might be expected, is correlated with triple-negative histology and poor prognosis [79, 80]. Aberrant beta-catenin expression is also correlated with lobular histology, given its association with E-cadherin loss [79, 80].

The absence of a clear targetable alteration made WNT an attractive but difficult pathway for drug development. Recently, casein kinase 1d (CK1d) was found to be amplified and overexpressed in strong correlation with WNT pathway genes in 36% breast cancers, particularly in luminal B and triple-negative ones. The CK1D inhibitor SR-3029 was highly effective in preclinical models (orthotopic cell line transplantation) [81].

4.2.3 Inducers of Epithelial-to-Mesenchymal Transition

Invasion of epithelial cells into connective and adipose tissue is a physiological phase of pubertal breast development and is governed by signaling pathways that have also been implicated in the acquisition of metastatic potential. This process bears resemblance to the physiological epithelial-to-mesenchymal transition (EMT) that occurs during crucial phases of embryogenesis like gastrulation. Whether tumoral invasion is truly an aberrant form of EMT has been a matter of dispute, mostly due to the fact that normal EMT implies dramatic morphological and molecular transitions that have not been consistently observed in breast and other tumors. However, a recent study showed that highly sensitive analysis of pathological specimens can identify cells with mixed epithelial/mesenchymal markers in invasive but not noninvasive breast cancers. These cells correlate with primary histology (mostly triple negative) and can be found circulating in proportions that vary according to treatment response [82].

The exact wiring of the signaling circuits responsible for EMT-like responses in breast cancer has not been fully worked out. Overexpression of specific individual transcription factors (SNAIL, TWIST, SLUG, and ZEB1/2) is able to initiate EMT and increase invasiveness in noninvasive breast cells [83–87]. Several extracellular signals are also implicated, most notably transforming growth factor beta (TGFb), WNT, and Sonic Hedgehog, the latter in turn activated by FOXC1/2 and the basal cell-specific p63 [88]. A common outcome of EMT response is the loss of E-cadherin expression, which results in a weakening of cell-to-cell adhesion and the release of a cytoplasmic pool of beta-catenin, which can now enter in WNT-dependent regulation. A second, recently discovered output of EMT activation is the activation of the Hippo pathway, which in breast cancer is correlated with metastatic behavior and resistance to chemotherapy [89, 90].

All these pathways are rarely affected by genetic aberrations in breast cancer but appear frequently deregulated through nongenetic mechanisms in poor-prognosis breast tumors, especially of the basal and claudin-low subtype [91, 92]. As such, they have attracted attention as drug targets [93] but are still limited to preclinical development.

4.2.4 GATA3, FOXA1, and Lobular vs Ductal Tumors

GATA3 and FOXA1 are both implicated in the regulation of estrogen-mediated transcription (see chapter by Magnani), and their expression is strongly associated with estrogen positivity in tumors [94]. If conditionally deleted during puberty or adult life, they abrogate or severely distort

mammary gland development, with loss of luminal cell identity in the case of GATA3 [95, 96] and a block in terminal end bud formation and invasion during puberty for FOXA1 [97]. Their functional similarities extend to their molecular mode of action, as both are so-called "pioneering" factors able to condition chromatin structure and subsequent binding of other transcription factors [98]. FOXA1, ER, and GATA3 physically interact with several other chromatin regulators in a "mega transcription factor complex" nucleated by the estrogen receptor in response to estradiol stimulation. As FOXA1 directly promotes ER expression, these three factors form a regulatory network able to stabilize estrogen-dependent transcription [99].

Intriguingly, the mutational pattern of GATA3 and FOXA1 has recently emerged as mutually exclusive in the two forms of strongly ER+ breast cancers: GATA3 is frequently mutated in ductal luminal cancers, while FOXA1 is as frequently mutated in lobular cancers [31].

Mutations in GATA3 are the third most common alteration in breast cancer globally [100]. SNVs are invariably heterozygous and cluster in three specific categories: splice site mutations at the junctions between exons 4/5 and 5/6 (20%), frameshift mutations in exon 6 (50%), and frameshifts in zinc finger 2 (10%). Also, GATA3 is frequently amplified (28% of all GATA3 alterations in the TCGA dataset), but this has received little attention. Mutation type 3 is the only type that has been characterized molecularly [101, 102]. Although SNVs cause apparent GATA3 loss of function, they appear to stabilize the non-mutated allele, leading to an intriguing model that can explain the requirement for maintaining heterozygosity [103]. The frequently mutated MAP3K1 is also a target of GATA3, and recently a germline variant in its GATA3-bound promoter was discovered in a genome-wide association study [104]. Loss of GATA3 expression correlates with acquisition of metastatic potential in the MMTV-PyMT mouse model of luminal cancer [105].

Mutations in FOXA1 cluster on lysines located on the wings of the Forkhead domain. These residues, when acetylated by EP300, prevent DNA binding; thus, their loss creates a strongly bound FOXA1 at sites of ER binding, amplifying a normally estrogen-dependent response on the absence of the hormone [31].

4.3 Chromatin Marks in Normal and Neoplastic Breast

The study of chromatin factors in breast development and cancer is probably less advanced than in other systems. In hematopoiesis and its malignancies, where mutations in chromatin factors were identified first, targeted drugs have already made it to the clinic and are routinely used [106]. We will skip lengthy discussions on basic chromatin structure, for which the reader is addressed to extensive reviews [107, 108] Proteins involved in interactions with chromatin have been functionally divided in writers, erasers, and readers [109], a useful classification that will be maintained here. We will focus on those aspects of chromatin regulation not specifically related to estrogen receptor biology, which is extensively covered by L. Magnani in this book (ref).

4.3.1 DNA Methylation

DNA methylation dynamics and the role of DNA methyltransferases in normal breast development have not been extensively investigated. In most breast tumors, primitive techniques could identify gross aberrations in DNA methylation as compared to normal tissues. Locus-specific analyses carried out at relevant genes (e.g., estrogen receptor) could also show aberrant methylation, but the relevance of this information has remained questionable; only recently genome-wide investigations have been systematically applied to large patient cohorts in the TCGA [100] and other studies [110, 111]. Unfortunately, even these systematic studies are complicated by the still poorly understood relationship with gene expression, and by significant heterogeneity in the techniques employed, none of which is truly able to fully cover all potentially methylated cytosines. In the TCGA, basal-like tumors tended to be globally hypermethylated, and, importantly, BRCA1 hypermethylation appeared to be a frequent mechanism (24%) for gene downregulation, potentially suggesting an involvement of BRCA functional loss in the absence of genetic alterations. A group of MSKCC performed a bioinformatically more refined analysis on 171 samples of heterogeneous histology and identified a cluster of tumors with methylation profile similar to that identified in colon cancer ("breast CpG island methylator profile," B-CIMP), which was associated with significantly lower propensity to metastasize. Different results were obtained by an Australian group focusing on triple-negative cancers; here, hypomethylated tumors were associated with better prognosis.

4.3.2 Histone Modifications

Histone modifications in normal breast cell populations have been systematically studied by Polyak et al. [112], who focused on the two marks that define actively transcribed and repressed genomic regions: trimethylation of H3K4 and H3K27. Regions where both signals overlap ("bivalent chromatin") are considered to be epigenetically plastic and

enriched in multipotent stem cells at genes with strong lineage-defining activity [113, 114]. $CD24^+$ and $CD44^+$ cells showed a different distribution of these marks, and many lineage-defining genes, especially transcription factors, were shown to maintain chromatin bivalency, suggesting a basis for phenotypic plasticity. In particular, ZEB transcription factors are bivalent in some tumor cell lines and regulate the expression of CD44, a marker of cancer stem cells with increased invasiveness. Robert Weinberg and colleagues demonstrated that cells in which ZEB1 is bivalent (but CD44 is repressed, like in luminal cancer cell lines) are able to resolve bivalency and lose the repressive H3K27me3 in response to TGFb, resulting in CD44 upregulation and acquisition of invasive traits [115], directly linking an extracellular stimulus with a chromatin-mediated phenotypic change. The Polyak lab investigated further the role of chromatin in phenotypic reprogramming. Using elegant cell fusion experiments between cell lines with luminal or basal features, they showed that the basal phenotype is dominant over the luminal and can be induced by even short-term exposure of luminal cells to basal cell total extracts; reprogramming correlated with the acquisition of epigenetic traits of the parental basal cell, in particular the super-enhancer profile defined by elevated H3K27ac [116]. It might be interesting to explore whether luminal-to-basal epigenetic reprogramming is at the basis of estrogen expression discordance between primary and relapsed tumors, which more often become estrogen receptor negative from positive than vice versa [117].

4.3.3 Chromatin Writers

Members of the Polycomb family (so called from the developmental phenotype observed in *Drosophila* mutants) are the best-studied chromatin writers in breast development. Polycomb proteins are organized in two sets of complexes which induce histone modifications associated with gene repression, namely, H2AK119 ubiquitylation (Polycomb repressive complex 1, PRC1) and H3K27 methylation (PRC2). Members of both complexes have been found to play a role in breast development and cancer [118–122]. Polycomb factors are involved in the maintenance of pluripotency in most if not all stem cells in adult and embryonal life; their genetic disruption leads to increased transcriptional plasticity at lineage-specific genes, resulting in a failure to coordinately execute differentiation programs. The ultimate outcome of Polycomb ablation is highly variable depending on the examined system and can result in cell death. Polycomb inhibitors, especially those directed against the PRC2 catalytic subunit EZH2 that is overexpressed and correlated with poor prognosis in breast cancer [123, 124], have shown responses in preclinical studies [125–127].

4.3.4 Chromatin Erasers

Factors that remove histone acetylation and methylation maintain chromatin in a dynamic state, making it more or less amenable to transcriptional changes. Many cancer cells depend on persistent deacetylase or demethylase activity for survival, and their ablation can lead to cell death or differentiation. Given their favorable chemical properties for drug design, chromatin erasers have been identified as interesting targets for drug development. Histone deacetylases (HDACs) were identified first as playing a role in breast cancer [128], especially by virtue of their negative effect on estrogen receptor expression [129]; thus, they have been mostly studied as sensitizers to endocrine therapy [130–132]. Of the several inhibitors with different degree of specificity synthesized so far, entinostat has reached the furthest clinical development, showing efficacy in a randomized phase II trial against placebo in combination with exemestane in aromatase inhibitor-refractory advanced ER+ breast cancer [133].

Recent research also revealed important roles for histone demethylases. The H3K4 demethylase JARID1B (also known as KDM5B), involved in mammary gland development and GATA3 recruitment to FOXA1 promoter [134], was found frequently amplified and overexpressed in multiple breast cancers, particularly in luminal cancers where overexpression of JARID1B target genes identified a subset of patients with poorer survival [135]. Another demethylase, LSD1 (also known as KDM1A), which targets H3K4 mono- and di-methylation and is involved in enhancer "decommissioning" during cell differentiation [136], was found to regulate breast cancer metastasis [137]. LSD1 inhibitors are in early clinical trials but have not been yet tested in breast cancers.

4.3.5 Chromatin Readers

Chromatin "readers" are proteins with domains able to recognize specific chromatin modifications and guide locus-specific assembly of transcription regulator complexes. A relevant example in breast biology is the Pygo2 factor that contains the PHD finger domain able to recognize H3K4me3. Pygo2 is a crucial transducer of WNT signals in breast development [138] and is essential for the survival of several breast cancer cell lines [139]. Disruption of chromatin interaction is a novel pharmacological strategy that has yielded intriguing results when targeted against bromodomain-containing proteins. These interact with acetylated histones and are important factors for super-enhancer activity. As super-enhancers are associated with highly tissue- or cancer-specific transcription [140], their targeting might benefit from an elevated therapeutic index. BET inhibitors, of which JQ1 is the progenitor, are undergoing rapid drug development and have recently shown particularly promising activity in triple-negative breast cancer [141].

Conclusions

Full understanding of the molecular pathways described in this chapter will require an elevated degree of integration between developmental biology, biochemistry, and epigenomics. The benefits that can be reaped for patients are high, as targeting differentiation and stem cells may lead to durable responses or even disease eradication, unachievable with drugs targeting proliferation or genome stability. Technological advancements of the last decade have made this endeavor realistic.

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Breast Cancer Microenvironment and the Metastatic Process

5

George Sflomos and Cathrin Brisken

5.1 Introduction

Breast cancer is the most frequently diagnosed cancer, the second leading cause of cancer-related death in women worldwide, and the leading cause of cancer-related death in less developed countries [1]. Overall prognosis is favorable with 85% survival chances in developed countries [2]. Most of the remaining 15% of patients succumb to sequelae of metastasis, the spread of cancer from one part of the body to another (Dictionary of Cancer Terms, NCI), as their disease becomes drug resistant. In poorer countries, women are more likely to die of their metastases because they are diagnosed at later stages of the disease and have less access to costly treatments [3–5].

In general, breast cancer cells that escape from the primary tumor take the lymphatic and/or venous route and home most frequently to the bones, the lungs, the brain, and the liver. The WHO distinguishes more than 20 different histologic subtypes [6], and global gene expression profiling has revealed at least 5 molecular subtypes of breast cancer [7–9]. Some of these subtypes have distinct clinical features and show different metastatic behaviors. Luminal/estrogen receptor-positive (ER^+) tumors disseminate more frequently to the bones than TN tumors do, and human epidermal growth factor receptor 2-positive ($HER2^+$) and TN tumors have a predilection for the brain [10]. Lobular carcinomas typically metastasize to the peritoneum and the ovaries [11].

Metastasis is a complex pathological process, and seven distinct steps have been defined at the cellular level. It begins with increased cell motility and cell migration followed by stromal invasion. Subsequently, tumor cells intravasate into lymphatic and/or blood vessels; they adapt to survive in the circulation; they extravasate, colonize distant organs, and eventually, sometimes after many years of dormancy, grow to overt metastases [12–15].

For decades, most research efforts concentrated on the cancer cell-intrinsic factors that drive this process. In recent years, the tumor microenvironment (TME) defined as the normal cells, molecules, and blood vessels that surround and feed a carcinoma (Dictionary of Cancer Terms, NCI) has moved to the center of attention. Both at the primary and at the metastatic sites, numerous noncancerous cells, cancer-associated specialized cell types, and matrix molecules interact with the tumor cells and affect their biological properties [16–18]. Heterotypic cell contacts, exosomes, cytokines, and other soluble factors produced by cancer and stromal cells are now known to support tumor initiation, progression, and metastatic spread [19]. In conventional breast cancer xenograft models, breast cancer cell lines are typically injected either subcutaneously or orthotopically in the mammary fat pad of immunocompromised mice [20]. Intriguingly, the site of injection affects metastatic capability with increased metastasis observed upon orthotopic engraftment [21, 22].

Throughout the multistep metastatic process, tumor cells rely on epithelial-mesenchymal transition (EMT) [12, 23]. This well-characterized process is repeatedly required during development as for the formation of the mesoderm from epithelial epiblasts during gastrulation, neural crest formation, and formation of muscle cell precursors from epithelial somite walls. In adulthood, it has a role in wound healing [12, 24–27]. All these processes have in common that epithelial cells dedifferentiate, lose cell adhesion, become more migratory, and acquire stem cell properties. Cancer cells hijack this developmental program to detach from the epithelial tissues of which they are part, to reach vessels, and to

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acquire the self-renewal properties and the cellular plasticity important in the metastatic process [28]. At the distant organ, the reverse process called the mesenchymal-to-epithelial transition (MET) is commonly found at metastases [27, 29]. Intriguingly, recent in vivo studies of murine tumors are arguing for a role of EMT and MET transitions in breast cancer drug resistance [30].

Here, we review how the cancer microenvironment affects the spread of cancer cells to distant sites and discuss how the microenvironment at the distant sites contributes to colonization and to the growth of the metastases. The important role of the normal tissue microenvironment as a barrier to tumorigenesis has been extensively described elsewhere [31]. Most of our insights into the metastatic process stem from experimental models, which do not distinguish between different clinical subtypes. Hence, wherever possible, we refer to clinical observations that provide clues of subtype-specific properties.

5.2 Cancer Cell: Nonautonomous Traits and Breast Tumor Microenvironment

Cancer-associated stromal cells react to the morphological and molecular changes the tumor cells undergo by epigenetic changes and alterations of their secretome [32]. The released factors can affect tumor progression directly and/or indirectly through recruitment of other cells, nonindigenous to the breast tissue. Examples are bone marrow-derived, mesenchymal stem cells and innate and specific immune cells, which in turn participate in stimulating a vicious cycle of cell-to-cell and factor-to-cell interactions.

5.2.1 Cell Types and Secreted Factors

5.2.1.1 Fibroblasts and Mesenchymal Stem Cells

The fibroblasts are the most abundant cell type in the breast stroma [33] and can both inhibit [31, 34] and promote tumor growth [31, 33–36]. During tumor progression, fibroblasts are converted cells to activated fibroblasts or cancer-associated fibroblasts (CAFs) by transforming growth factor β1 (TGFβ1) [37], Wnt7a, and other factors secreted by the tumor cells [33, 38, 39]. CAFs are characterized by high-level expression of fibroblast-specific protein 1 (FSP1), fibroblast-activating protein (FAP), alpha-smooth muscle actin (α-SMA), and TGFβ1 [40]. They orchestrate and promote the metastatic process in two ways; first, they induce EMT through activation of TGF-β receptor signaling and extracellular matrix (ECM) remodeling [34]. Second, they recruit innate and specific immune cells to the TME and subsequently activate them. The activated immune cells, in turn, stimulate the metastatic potential of cancer cells [18, 33, 35]. More specifically, CAFs release immune-modulatory molecules including interleukins, interferons, and tumor necrosis factor α (TNFα) to attract macrophages. The tumor-associated macrophages (TAMs) are the dominant portion of the leukocyte population within the tumor [41]. They can modify the cancer cell phenotype generally leading to a more aggressive behavior in breast cancer, through factors that have not been clearly identified yet, but hypoxia has been implicated [42]. TAMs in turn, at least in models of skin carcinoma, support tumor angiogenesis [39]. Moreover, CAFs recruit regulatory T cells (Tregs) through the secretion of various chemokines including CCL5, as shown in GEMMs of mammary carcinogenesis [43].

Experiments in the TN MDA-MB-231 xenograft model showed that CAFs control organ specificity of metastases by secreting two cytokines that are mainly expressed by stromal cells in the bone marrow, the stromal cell-derived factor 1 (SDF1/CXCL12) and the insulin-like growth factor 1 (IGF-1) [44, 45]. Activation of the cognate receptor, CXCR4, on the cancer cells activates AKT thereby increasing tumor cell survival in the bone [46]. Bioinformatic analysis of primary tumor gene expression profiles revealed that Src activity correlated with late bone metastasis, and Src activity was shown in the MDA-MB-231 model to indeed enhance tumor cell survival in the bone by facilitating CXCL12-CXCR4-AKT signaling and by increasing resistance to TRAIL-induced cell death [46].

Mesenchymal stem cells (MSCs) are recruited to damaged and ischemic tissues. More recently, they were also shown to be drafted to the TME following calls by numerous factors such as growth and angiogenic factors as well as chemokines and ECM proteases, all released by cancer and stromal cells as reviewed in [47, 48]. The MSCs present in invasive human breast carcinomas can be differentiated into various cell types including fibroblasts and pericytes, and their effects on tumor growth are complex. They were shown to promote tumor growth and angiogenesis through elevated SDF1 secretion [49]. Experiments in subcutaneous breast xenografts showed that once recruited to the TME, MSCs in turn secrete the chemokine CCL5, which increases the motility and invasiveness of the cancer cells, which express the cognate receptor CCR5, and promote lung colonization [50].

5.2.1.2 Adipocytes

Another prevailing cell type in the TME is the adipocyte that also interacts in different ways with the tumor cells [51, 52]. Analyses of human clinical samples and *in vivo* experiments with mice have shown that adipocytes are frequently found at the invasive front of human breast tumors. Here a dialogue between the cancer cells and adipocytes occurs; the invading tumor cells are able to modify the adipocytes,

which in turn stimulate cancer cells to a more aggressive phenotype [53].

Furthermore, the fat cells promote tumor progression through systemic effects by secreting hormones, such as oestradiol (E2) and prolactin, as well as paracrine factors like the major fat tissue-derived adipokines such as leptin and adiponectin which promote breast cancer progression through activation of proliferation and survival [54–56]. A series of in vitro coculture and in vivo experiments suggest that secretion of interleukin-6 (IL-6) by adipocytes makes tumor cells more invasive and increases their metastatic potential [53]. Similarly adipocytes promote breast cancer progression through secretion of pro-inflammatory cytokines such as TNFα, which increase stem cell numbers and enhance metastasis in cell coculture and animal models [57, 58].

In this regard, the tumor-promoting effects of obesity, which are observed in postmenopausal women, relate to increased levels of E2 and of pro-inflammatory cytokines released by the adipose tissue [59, 60]. Elevated systemic E2 levels result from increased aromatase activity not only the adipocytes of the TME but in various fat depots in the body. To what extent these effects are important for the metastatic process systemically versus locally remains to be teased apart experimentally. Interestingly, when human breast adipocytes from obese patients were grafted to immunocompromised mice to model the inflammatory environment of the human breast, the mouse tumors were enriched with adipocytes secreting CCL2/IL-1β. This recruited macrophages, which in turn stimulated CCL2-associated angiogenesis [61].

5.2.1.3 Vasculature

The vasculature in the TME is another key player in the instigation of metastasis. It consists of an inner layer of endothelial cells, pericytes that wrap around the endothelial cells, and, in the case of larger vessels, smooth muscle cells. During tumor progression, the normally quiescent vasculature is activated by VEGF released by tumor and stromal cells, and new vessels sprout to sustain tumor growth [12, 62]. Critical to the metastatic process is vessel integrity, which deters cancer cell migration and prevents tumor spread into the circulation. Pericytes are responsible for vessel integrity. Consistently, many studies in mouse models have shown that low pericyte coverage is associated with invasive breast cancer, decreased survival, and lung metastasis [63–65].

5.2.1.4 Immune Cells

It was realized a long time ago that the white blood cells, which constantly patrol normal breast tissue, also closely interact with breast tumor cells [66]. Their role is ambiguous; frequently, differentiated tumor-infiltrating immune cells and "tumor-educated" macrophages further the multistep metastatic cascade by promoting tumor cell invasion, intravasation, and their survival in the bloodstream. They also assist in tumor cell arrest, extravasation, and overt growth at metastatic sites [67]. In specific clinical scenarios, however, an immune cell infiltrate is indicative of a good prognosis [68].

Studies with the MMTV-Erbb2-transgenic mouse mammary carcinoma model [43] showed that tumor-infiltrating regulatory T cells promoted lung metastasis through expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), a protein implicated in epithelial cell proliferation of the normal breast [69]. Consistently, RANKL overexpression stimulated the metastatic progression of RANK-expressing and HER2-overexpressing mammary tumors [43, 67].

Recent studies in a mouse model of invasive lobular carcinoma generated by targeted deletion of E-cadherin and p53 in the mammary epithelium indicate that neutrophils induce the release of several cytokines in the TME that increase lung metastasis without affecting primary tumor growth [70, 71]. Moreover, neutrophils support lung colonization of metastasis-initiating tumor cells in the metastatic MMTV-polyoma middle T antigen (PyMT) mammary tumor mouse model [72].

Initially, macrophages were implicated in the antitumor immune reaction [73, 74]. However, recent studies indicate that TAMs promote angiogenesis, cell migration, invasion, and intravasation, thereby increasing the propensity of the cells to leave the primary site [75]. In the PyMT mammary tumor model, macrophage infiltration is seen early during tumor development when hyperplasias are present [76]. The recruitment of TAMs into TME is principally regulated by cytokines, chemokines, and growth factors secreted by both tumor and stromal cells [77]. In breast cancer patients, TAMs abundantly produce the chemokine CCL18, and its expression in cancer stroma and blood is associated with increased metastasis and reduced patient survival [78]. Mechanistically, CCL18 promotes the invasiveness of cancer cells by triggering integrin clustering and enhancing their adherence to extracellular matrix [78].

5.3 Breast Tumors in Transient and Metastatic Microenvironments

Breast cancer cells enter the lymph vessel in the TME and ultimately reach the blood vessels. Anytime after intravasation in the bloodstream, they may encounter antitumor immune cells and metabolic and oxidative stress and be exposed to extensive shear forces in the bloodstream. Once in the vessels, circulating tumor cells (CTCs) need to exit from the lumen of blood and/or lymphatic vessels and penetrate to the distant tissue. This process is highly inefficient with an estimated 1 out

of 10,000 cells successfully extravasating and homing to a distant site [16, 79, 80]. Many different cell types can be found in the blood including immune cells, fibroblasts, and platelets with important roles in the metastatic process [32].

5.3.1 Platelets

Platelets have emerged as essential "protective" escort of the carcinoma cells on the move; they appear to constitute a special type of mobile "local" microenvironment [81, 82]. Physiologically, platelets are activated when the continuity of the endothelial layer is disrupted and the underlying subendothelial matrix is exposed [83]. In the breast tumor setting, they can be activated through physical contact with tumor cells [84]. At the primary site, platelets along with macrophages and MSCs contribute to EMT of the cancer cells. In the bloodstream, platelets have dual role in the fitness of disseminated breast tumor cells both through direct physical contact and through secretion of paracrine factors both of which increase tumor cell survival and extravasation by maintaining or/and inducing an EMT status [84]. In vivo experimental evidence indicates that the paracrine effects can be ascribed to the secretion of bioactive growth factors stored in α-granules, such as EGF, PDGF, TGFβ, and VEGF, as well as inflammatory cytokines and chemokines also stored in α-granules [84, 85]. Within the peripheral tissues, activated platelets contribute to the recruitment of monocytes and granulocytes thereby helping to establish pro-metastatic and metastatic niches [83, 85, 86].

5.3.2 Metastatic TME (MTME)

Metastatic sites are generally considered inhospitable microenvironments and present a challenge to cancer cell fitness and survival [16]. Consistently, many of the CTCs that successfully extravasated from the bloodstream to a distant tissue remain dormant for months or, in the case of ER^+ tumors, even decades. Only some get reactivated and go on to form clinically apparent tumors. Several lines of evidence indicate that secreted factors, produced by tumor and stromal cells in the TME, promote the progression of tumor-specific organ colonization. Heparanase endoglycosidase secretion by primary breast tumors promotes bone resorption in a GEMM model [87]. Furthermore, tumor cell-derived exosomes may represent the postal code on a letter and lead to distinct organ colonization depending on the integrins they carry [88]. Noteworthy, the microenvironment at the distant site can induce epigenetic changes that enhance proliferation and reduce apoptosis of the metastatic cancer cells as was elegantly illustrated by xenograft mouse models [89]. Intriguingly, metastatic breast cancer cells that have lost phosphatase and tensin homolog (PTEN) are primed for the brain most probable through the induction of the chemokine (C-C motif) ligand 2 (CCL2) [89]. Moreover, immunohistochemical analysis of clinical samples for PTEN and CCL2 revealed significantly higher CCL2 expression in brain metastases than in matched primary tumors. Mechanistically, epigenetic regulation is implicated; astrocytes secrete exosomes that cause adaptive PTEN cancer cell loss [89]. Moreover, metastatic cancer cells can directly interact with stromal cells. This heterotypic dialogue with the distant stroma can provide support to the cancer cells at the early steps of colonization and promote metastasis as nicely illustrated experiments in a xenograft model of intrailiac artery injection [90]. In this model, the ER^+ breast cancer cells, MCF7, colonized efficiently the bones and made physical contacts with osteogenic stromal cells through adherens junctions. This led to increased cell proliferation of cancer cells and growth of metastasis through the activation of mTOR pathway [90]. On the other hand, cancer cells activate bone cells. For example, bone metastatic breast cancer cells promote osteolytic lesions by stimulating the formation and activity of osteoclasts via production of colony stimulating factor 1 (CSF1) as well as parathyroid hormone-related protein (PTHRP) and tumor necrosis factor-α (TNFα) [91]. The secretion of these cytokines and hormones activates the RANK pathway and inhibits the synthesis of the osteoprotegerin, thereby increasing the number and activity of osteoclasts. Finally, xenograft experiments have revealed ER^+ breast cancer-mediated systemic instigation by supplying circulating platelets with pro-inflammatory and pro-angiogenic proteins, supporting outgrowth of dormant metastatic foci [92].

5.3.3 Hypoxic Conditions in the TME

As tumors grow, lack of adequate oxygen supply leads to hypoxia. Hypoxic breast cancer cells are more prone to invade and metastasize, and they also respond less efficiently to drug treatment [93]. Central in the cellular response to hypoxia is the transcription factor hypoxia-inducible factor 1α (HIF1α); it upregulates cytokines, extracellular matrix proteins, and secreted proteins such as lysyl oxidase (LOX).

The HIF1α target LOX is highly expressed in primary breast tumors and is significantly associated with metastasis in ER^- patients [94]. Moreover, LOX is a critical mediator of bone marrow cell recruitment during the formation of the pre-metastatic niche [95]. HIF1α facilitates the initiation of EMT by inducing the expression of the master mesenchymal regulator, the transcription factor TWIST [96]. Hypoxia also triggers the release of exosomes. Studies using the 4 T1-BALB/c syngeneic animal model of metastatic mammary carcinoma showed that LOX activates normal bone-constituent cells called osteoclasts, which in turn enhance bone breakdown, favoring the formation of a pre-metastatic niche where disseminated circulating breast cancer cells find the appropriate microenvironment to form metastasis [94]. Preparation of the niche [97] is a key regulator of angiogenesis [98].

5.4 Cancer Cell-Autonomous Traits

5.4.1 Genetic Aberrations

Tumor cell-intrinsic factors that contribute to metastasis include genetic and epigenetic alterations with the ensuing changes in gene expression and biological properties. Numerous somatic mutations, gene fusions, gene amplifications, and cancer predisposing single-nucleotide polymorphisms are well documented in breast cancer [55, 99–102]. Some of these are thought to drive tumorigenesis by bestowing essential tumor cell properties on normal cells [12]. However, despite many efforts to identify mutations specific to metastases that could trigger a metastatic switch, evidence for such failed to come forward. Hence a model began to prevail in which the ability to metastasize is inherent to the primary breast tumor. In line with this view, gene expression signatures were identified in primary tumors that are associated with higher likelihood to metastasize [14, 103–105].

Recent findings, however, have challenged this view. Advances in sequencing technology have led to the realization that tumors can be composed of a myriad of different subclones [106], and xenograft models combined with DNA bar coding technology have revealed unexpected dynamics during tumor evolution [107]. With the in-depth comparisons of DNA sequences from primary tumors and their matched metastases, examples of metastasis-specific mutations are beginning to emerge. The most prominent example is mutations in the estrogen receptor α (*ESR1*). They were originally identified two decades ago by S. Fuqua and colleagues who screened metastatic samples for *ESR1* mutations [108] but largely dismissed as a very rare event. This changed when deep sequencing revealed that they occur in as many as 32% of metastases [109] and in as many as 42% of circulating tumor DNA (ctDNA) samples from women with metastatic ER$^+$ disease who had been treated with aromatase inhibitors for their metastatic disease [102]. In primary breast carcinomas, these mutations either failed to be detected at all or were found in less than 0.6% only [102, 109, 110]. Most of the mutations are found at the C-terminus in the ligand-binding/AF2 domain and lead to estrogen-independent growth in vitro [111]. This suggests that metastatic cells require constitutively active ER signaling for the survival and growth in the metastatic microenvironments in the absence of estrogens.

Recently, whole-exome sequencing of 86 brain metastases and matched primary tumors including 21 breast tumor samples identified metastasis-specific mutations [112]. Specifically, analysis of breast cancer brain metastases revealed that 47% of genetic aberrations were not detected in the same patient's primary tumor. Patients with HER2-amplified breast cancer who developed brain metastasis under trastuzumab showed amplification and activating point mutations in epidermal growth factor receptor (*EGFR*) (L858R) and fibroblast growth factor receptor (*FGFR1*) amplification specifically in the metastatic sample but not in the primary tumor DNA. The findings from this study are of important clinical implications, as both the *EGFR* and the *FGFR* mutations are druggable. The mutations that appear only in the metastatic sites may relate to the therapeutic responses and the way the cancer cells interact with the TME [113].

Mutations in GATA binding protein 3 (GATA3) interfere with its DNA-binding ability, reducing or diminishing its binding, and are commonly found in NST luminal-like molecular subtype in human breast cancers [100] with high frequency (13%) [99, 114]. Moreover, loss of GATA3 expression in breast tumors has been linked to aggressive tumor development, poor patient survival, and increased metastatic potential [115–119]. In a spontaneous metastasis experimental model using the LM2 lung-tropic breast cancer cells that were derived from MDAMB231, increased expression of GATA3 specifically inhibited metastasis to the lungs without affecting extravasation [116]. Mechanistically, GATA3 induces *microRNA-29b* expression, which in turn inhibits metastasis by targeting a network of pro-metastatic regulators involved in angiogenesis [115].

Mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA)* are found in 40% of ER$^+$ breast cancers. A study of 292 clinical samples derived from independent international cohorts analyzed for the presence of mutations showed that they are correlated with lymph node metastasis suggesting that PI3K/AKT activation may enhance invasion of cancer cells to lymph nodes [120]. Of note, *BRCA1* germline mutations, which typically lead to aggressive breast cancers, have been linked to increased probability of cerebral metastases [121]. In particular, 67% ($n = 15$) of *BRCA1* mutation carriers were found to develop metastases in the brain compared to 0 and 6% of *BRCA2* mutation carriers ($n = 12$) and noncarriers ($n = 58$), respectively.

Mutations in genes that are implicated in metabolic processes have recently been identified and amplification of genes encoding for metabolic enzymes has been found in breast cancers. An example is the gene encoding phosphoglycerate dehydrogenase, which is frequently amplified in TN breast cancers and increases flux through the metabolic pathway of serine/glycine synthesis thereby providing advantages for bone metastatic breast cancer cells because cell proliferation is stimulated and osteoclastogenesis enhanced [122, 123]. Moreover, the proto-oncogene Neu product (ERBB2) stimulates glycolysis by AKT1-dependent and AKT1-independent pathways. Interestingly, in many breast cancer cell lines, overexpression of ERBB2, a hallmark of HER2$^+$ tumors, leads to increased glucose uptake and lactate production and decreased oxygen consumption [124]. Whether this contributes to the increased propensity of HER2$^+$ tumors to metastasize to the brain remains to be explored. It remains also to be seen how metabolic changes are related to metastasis and which are the exact molecular mechanisms that give advantages to the cancer cells to access vessels and survive in the bloodstream and establish metastases.

5.4.2 Noncoding RNAs (ncRNAs)

Over the past years, ncRNAs have emerged as important mediators in the crosstalk between breast cancer cells and their microenvironment [125]. During breast cancer progression, expression of particular small, (miRNAs) is deregulated. When human breast cancer cell lines were injected into the tail vein of immunocompromised mice, seven miRNAs, miR-335, miR-126, miR-34a, miR-31, let-7, miR-200 s, and miR-29b, suppressed—whereas miR-10b, miR-373/520c, miR-200 s, miR-21, miR-9, and miR-103/107 promoted—several steps of the metastatic process [125, 126]. In particular, loss of expression of tumor suppressor miRNAs through epigenetic silencing lead to increased brain and lung metastases [126, 127]. Mechanistically, silencing and/or overexpression of specific miRNAs affects the expression of numerous genes, including genes involved in EMT, endothelial recruitment, anoikis resistance, invasion, and colonization. For example, silencing of miR-200 s triggers EMT via ZEB1/2-dependent repression of E-cadherin upregulation or miR-21 upregulation which is correlated with active mTOR and STAT3 signaling and increased invasion, tumor growth, and survival. miR-29, which inhibits breast cancer metastasis, for example, targets genes with established roles in collagen remodeling and angiogenesis such as VEGFA, LOX, MMP2, ANGPTL4, and PDGF. Interestingly, micro-vesicles secreted by tumor cells are enriched for miR-9 and have a direct impact on stromal cells through the regulation of the endothelial cell migration.

5.5 Conclusions and Future Perspectives

In vitro and in vivo studies have significantly advanced our understanding of the metastatic process and identified numerous pathways and molecular mechanisms that control it (Fig. 5.1). However, some limitations of the models need

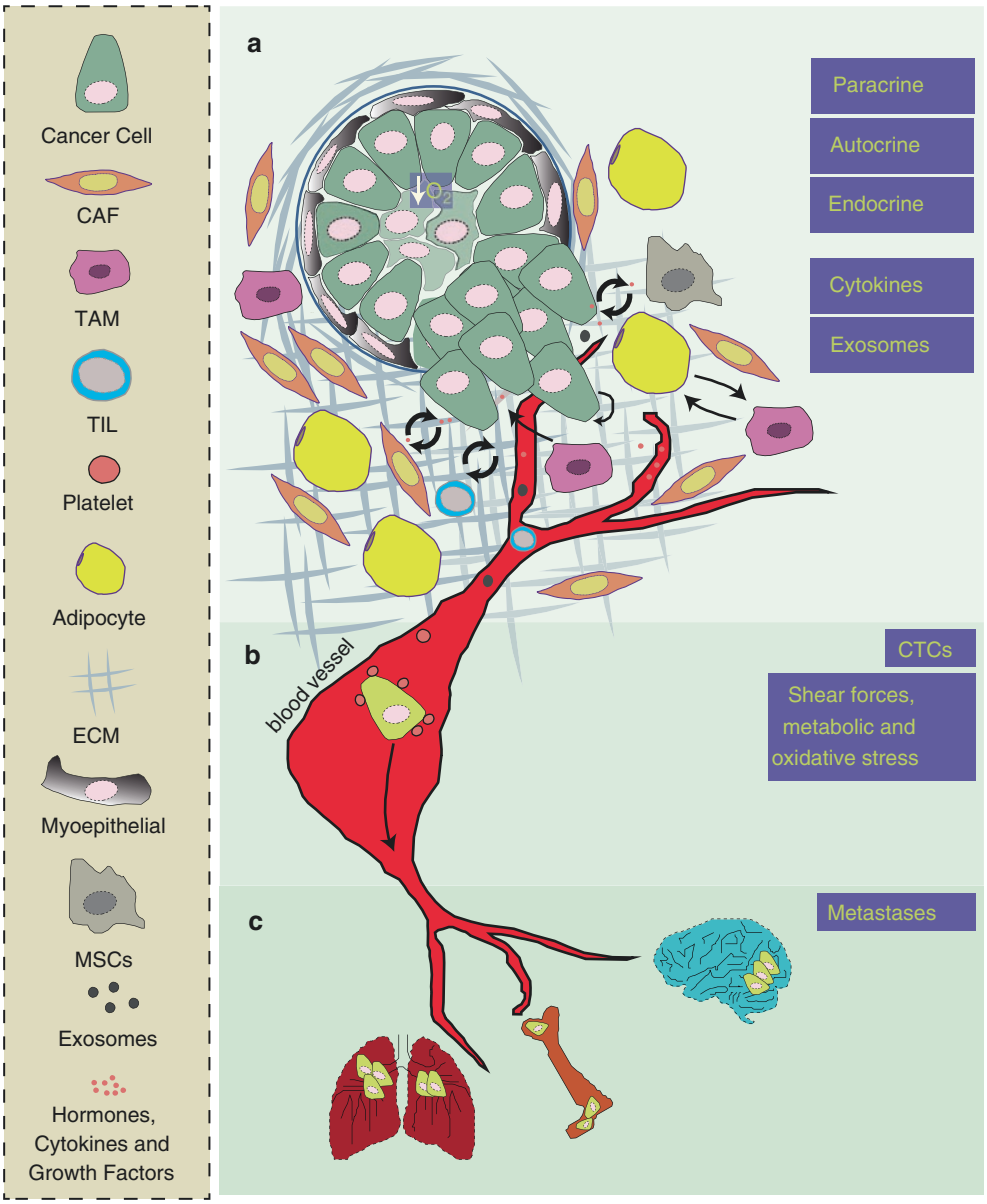

Fig. 5.1 Interactions of breast cancer cells with stromal cells and molecular factors of the tumor microenvironment: **a** Cells and molecular factors found at the primary breast tumor microenvironment and their heterotypic interactions, **b** Circulating tumor cells (CTCs) that have detached from the primary tumor travel in the bloodstream and constitute potential "seeds" for metastases, **c** Breast cancer cells that escape from the primary tumor and survive in the bloodstream frequently colonize and potentially can form metastases to distant organs

to be considered as we try to extrapolate the experimental findings to the clinical situation. The widely used animal models fail to fully reflect the heterogeneity of breast cancer with its many different histopathological and molecular subtypes. It cannot be excluded that some of the factors implicated in xenograft models and GEMMs of a particular subtype may have different roles in different breast cancer subtypes. In particular, a lot may remain to be learned about the hormone-dependent tumors because there are few preclinical models for the ER$^+$ subtypes [128]. GEMMs are mostly ER negative, and few ER$^+$ breast cancer cell lines grow as xenografts. We also need to consider that widely used xenograft models, in which large numbers of cells are injected either subcutaneously, directly into the bloodstream, or to a distal organ, create artifacts that affect the interpretation of the results, and they fail to recapitulate the complete metastatic process. The few ER$^+$ cell lines that grow in vivo need to be provided with exogenous E2 [129]. This creates a nonphysiological systemic environment, which in turn impinges on the local microenvironments. A study with mice that had been xenotransplanted with MCF7 cells and were first hormone depleted by ovariectomy and subsequently hormonally stimulated suggests that ER$^+$ micrometastases are exquisitely sensitive to E2 and progesterone [130]. We have recently demonstrated that the microenvironment is a determinant of the luminal phenotype; ER+ tumor cells grow well in the absence of exogenous hormones when they are engrafted into the milk ducts. Interestingly, in this model MCF-7 cells metastasize to the bones as well as lungs and brain [131]. As such it will be interestign to study the metastatic process in this new model.

Hence more complex models are necessary to improve our knowledge on heterotypic interactions and paracrine signaling and elucidate the role of numerous significant factors such as ECM stiffness and mechanical forces in the TME. 2D coculture, 3D coculture, organotypic slice culture, and in vivo findings need to ultimately be validated in patients.

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Mouse Models of Breast Cancer: Deceptions that Reveal the Truth

6

Joana Pinto Couto and Mohamed Bentires-Alj

6.1 Introduction

Breast cancer is the most frequently diagnosed malignancy and results in the highest cancer mortality in women aged 20–59 years worldwide [1]. The disease usually progresses from hyperplasia to ductal carcinoma in situ (DCIS), and subsequently invasive carcinoma and metastasis, the latter accounting for almost all deaths among these patients [2].

Breast cancer is not a single homogeneous disease but rather a collection of distinct phenotypes that can be distinguished using clinicopathological parameters such as receptor status [estrogen receptor (ER), progesterone receptor (PR), and/or ErbB2/HER2] [3]. These parameters have a prognostic value and guide the selection of therapies. For example, most triple negative breast tumors (ER, PR, and ErbB2/HER2-negative), which occur in around 20% of breast cancer patients, present the worst prognosis. Unlike patients with ER-, PR-, and ErbB2/HER2-positive tumors, who receive antihormone or anti-HER2 therapies, targeted therapy for triple negative breast cancer is still lacking. While some patients with metastatic disease initially respond to therapy, curative strategies for the majority are not available.

In early 2000, seminal studies using molecular profiling segregated breast cancers into normal-like, luminal A, luminal B, HER2-enriched, claudin-low, and basal-like [4–6]. These subtypes are associated with clinical prognosis [4, 7–10] and possibly response to therapy [11]. Such studies were complemented later by integrated genomic and transcriptomic analyses that revealed additional subgroups with distinct clinical outcomes and also increased awareness of tumor heterogeneity [12–17]. Heterogeneity is found among tumors from distinct patients (interpatient heterogeneity), within primary tumors (intratumoral heterogeneity) or metastatic lesions (intrametastatic heterogeneity), and among different metastases of the same patient (intermetastatic heterogeneity) [18]. These taxonomic studies paved the way to ongoing research that attempts to rationalize the genomic alterations found in cancer cells and to leverage this knowledge for improved therapy of patients with breast cancer.

"A model is a lie that helps you see the truth," according to American oncologist Howard Skipper. Indeed, mouse models have been crucial to our understanding of the etiology of cancer and its dependencies, as well as to the validation and advancement of drug candidates in the clinic [19]. In this chapter, we discuss mouse models used in breast cancer research, in particular those that have shed light on the mechanisms of the disease and have assisted in the identification and/or validation of therapeutic targets. We will elaborate on the earliest models and then discuss transplantation-based models and finally transgenic mice.

6.2 The Earliest Models: Spontaneous and Carcinogen-Induced Mammary Cancer

Detailed narratives of mouse models of breast cancer from the mid-nineteenth century up to recent years have been elegantly provided by R. Cardiff and D. Medina [20, 21]. The first studies of mouse mammary cancer were performed on spontaneous tumors, mostly those involving the mouse mammary tumor virus (MMTV) or other oncogenic viruses that trigger mammary cancers in certain inbred mouse models [22]. In 1982, R. Nusse and H. Varmus discovered that MMTV is an insertional mutagen that activates transcription of proto-oncogenes located near its integration sites [23]. Several of these insertion sites were later found to include members of the *Wnt*, *Fgf*, and *Rspo* gene families, as well as *eIF3e* and *Notch4* and others [24–26]. Following the development of transgene technology, the MMTV-LTR

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(long terminal repeat) has been used up to this day to drive gene expression in the mammary gland of over 50 transgenic mouse models of breast cancer [22].

Besides viruses, mammary tumors can also be induced by treatment with various chemicals (reviewed in [20]). The most frequently used chemical carcinogens have been 3-methylcholanthrene, 7,12-dimethylbenzanthracene (DMBA), and urethane, of which DMBA is the most potent, although its effect on mice is strain dependent. Since most of these chemical carcinogens are not significant etiological agents for human breast cancer, their use in research has declined over the years. In fact, radiation is the only well-documented environmental carcinogen in the mammary gland, and irradiated mouse mammary glands are considered to be a relevant model for carcinogen-induced human breast cancer [27, 28].

Hormones also contribute to mammary cancer. One of the earliest indications of the importance of hormones for mammary tumorigenesis dates back to an observation in 1896 that removal of the ovaries from a woman with breast cancer caused tumor regression [29]. Further experiments showed that ovarian estrogens contribute to breast cancer initiation and progression [30], paving the way for the development of agents that block estrogen signaling (e.g., tamoxifen). Further evidence of the importance of hormones in mammary tumorigenesis is that continuous exposure to prolactin induces mammary tumors in mice [31].

6.3 Transplantation Models of Breast Cancer and Metastasis

Tumorigenesis and metastasis are multistep processes involving profound interactions between transformed cells and the surrounding environment, for example, immune cells. Cancer immunoediting has been proposed as a mechanism of such interactions in which the immune system eliminates abnormal antigen-expressing cells during tumorigenesis (a process termed elimination). This may result in an equilibrium state in which tumor cells that escaped elimination remain under the influence of the host immune system. However, cells may eventually escape this immune surveillance and lead to overt tumor development and metastatic dissemination [32].

The fatal hallmark of breast cancer is its ability to metastasize [33, 34]. During metastasis, cancer cells penetrate the basement membrane and reach blood or lymph vessels (intravasation) (1) survive in the circulation (2) extravasate into secondary organs (3) where they may remain dormant (4) or proliferate and colonize the organ [35]. Clearly, no single approach can model all aspects of this complex process, and several transplantation-based models and techniques have been developed to study the metastatic cascade. The pros and cons of each approach can be examined, and those appropriate to the question of interest can be applied. The operation of a functional immune system in the recipient mouse model (syngeneic model) or its deficiency (xenograft model), the use of ectopic or orthotopic transplantation, and the transplantation of a tumor fragment or cancer cells or a primary tumor-derived xenograft will all have paramount consequences for mammary tumor development, progression, metastatic tropism, and growth.

6.3.1 Transplantation in Immunocompetent Versus Immunodeficient Animals

The microenvironment contributes significantly to breast tumorigenesis. Given that some secreted factors lack cross-species mouse-human reactivity [36], the transplantation into syngeneic hosts of mammary cancer fragments or cells from carcinogen-induced tumors or from genetically engineered mice is crucial to the study of tumor-host interactions. Syngeneic models are very important for dissecting cross talk between cancer cells and the immune system, particularly given the importance of the latter in tumor progression and metastasis. They are equally important for the study of antitumor immunotherapy.

Inbred mice, however, do not recapitulate the complexity and diversity of human genomes and may display mouse-specific oncogenic idiosyncrasies. To avoid these potential limitations, human cancer cells can be transplanted into immunodeficient mice; a method referred to as xenograft transplantation. The most common mouse strains used for the implantation of human cells are *nu/nu* (nude), *Rag1/2* knockout (KO), SCID, SCID-beige, NOD-SCID, NOG, and NSG. These differ in the severity of their immune defects [37], angiogenesis, and metastasis susceptibility [38]. As discussed above, tumor-host interactions may be limited by species boundaries, and the absence of the immune system precludes the investigation of this major contributor to tumor surveillance and progression to metastasis when human cells are transplanted into immunodeficient mice. The development of humanized models may circumvent this potential drawback [37].

6.3.2 Sites of Transplantation: Ectopic Versus Orthotopic

With the generation of the first inbred mouse strain (DBA) in 1910 by C.C. Little and the development of subsequent inbred strains [39], transfer of cells from one organism to another and the growth of explants in a precise and reproducible manner were finally possible [20]. Mammary cells and tumors are transplanted in two ways: orthotopic and ectopic.

In *orthotopic* transplantation, cells are implanted at the site of the initial tumor. This technique has been used extensively in breast cancer research, given the easy accessibility of the mouse mammary gland. In 1959, DeOme and his student Faulkin developed cleared fat pad transplantation, the signature technique of the mammary gland field [40]. This consists of removing the rudimentary mammary ductal tree of a 3-week-old recipient female mouse. Normal or neoplastic mammary gland fragments or cells from a donor mouse are then implanted into the resulting epithelium-free fat pad. This assay has proved valuable for studying the biology of the normal and neoplastic mammary gland, e.g., of stem cell potential, tumor initiation, and the contributions of stromal and epithelial cells to these processes [20]. Orthotopic transplantation is particularly useful when applied to transgenic mice that develop other neoplasias prior to the onset of mammary tumors, for example, in the case of p53-null mice [41]. Transplantation of p53-null preneoplastic cells into syngeneic mice was found to be sufficient to promote mammary tumorigenesis [41, 42], and this was further enhanced by hormonal stimulation [41]. Orthotopic transplantation can also be performed without removal of the mammary ductal tree, especially when the sources of the explants are tumor cells or fragments. This method may result in more metastases than ectopic transplantation [38] and is referred to as the "spontaneous metastasis model." Recently, the team led by D. Medina has developed a variation of the orthotopic method by injecting breast cancer cells intraductally. This method is effective for studying the progression from DCIS to invasive carcinoma [43, 44].

One problem that can arise when transplanting cancer cells is that before the animals develop metastases, the tumors grow to such an extent that the experiment must be terminated for ethical reasons. This is common with fast-growing cancer cell lines. To overcome this obstacle, tumors can be resected, allowing more time for metastases to develop. This approach can be particularly useful in overall survival studies, which should be the main readout of the success of treatments in preclinical models. In fact, it has been shown that primary tumor metrics do not necessarily predict metastatic behavior and disease progression in mice [45, 46] (Amante R, Leroy C, and M.B-A., unpublished data).

Cancer cells can also be injected ectopically into the highly vascularized renal capsule or subcutaneously, although these sites do not mimic the original microenvironment of breast tumors. Injection of cancer cells directly into the circulation system or at a given metastatic site is often referred to as the "experimental metastases method." Injection into the systemic circulation can be performed via the lateral tail vein (the preferential colonization site is the lung), the intraportal and intrasplenic veins (liver), intracarotid (brain), intraperitoneal (local invasion), intracardiac (bone, brain, adrenal glands, liver, and ovaries), or intra-tibial (bone) [47]. Once in circulation, cancer cells may be trapped in capillaries of the organ, extravasate, and eventually colonize the foreign site. The method is fast and allows control of the number of cells that reach the circulation. Moreover, given that breast tumor relapse in patients often occurs at metastatic sites, experimental metastasis is a very useful preclinical approach for rapidly assessing the effects of therapies. However, the method is less informative about the early steps of metastasis, when breast cancer cells undergo stringent selection. Moreover, when single cells are injected, the contribution of emboli and tumor clusters to metastasis cannot be evaluated [48]. Nevertheless, these models have been instrumental in the discovery of important aspects related to organ tropism of breast cancer cells. Good examples come from the work of J. Massague and team, who generated and studied organ-tropic (brain, bone, and lung) metastatic breast cancer cells by systematic reinjection of cells into the systemic circulation after their isolation from the metastatic organ [49–54].

6.3.3 Cancer Cells, Tumor Fragments, and Primary Derived Xenografts

Human and mouse mammary cancer cell lines have proved useful for investigating several aspects of mammary tumorigenesis. However, as most of these cell lines were derived from pleural effusions and were highly passaged in vitro, clones may have been selected that do not necessarily recapitulate fundamental features of the parental tumor, including tumor heterogeneity [55–58]. To avoid this issue, cell suspensions or fragments derived directly from human breast tumor biopsies can be transplanted subcutaneously or into the mammary fat pad of immunodeficient mice; these models are referred to as "patient tumor-derived xenografts" (PDXs) [59–61]. PDXs are thought to be stable and to resemble the characteristics of the original tumor, such as heterogeneity, histopathology, molecular subtype, metastatic potential, and/or drug sensitivity [59, 62–64]. However, they are subjected to selection pressure when grown in mice, and it seems that only the more aggressive, less-differentiated clones of the tumor grow as PDX [65, 66]. A further study found that PDXs resemble the genetic landscape of the metastases more closely than that of the primary tumor [67]. PDXs have also been described that result in lung metastases in mice although they originated from patients with no signs of lung metastases [63]. In the era of personalized medicine, PDXs could be very useful for testing the efficacy of therapies and predicting mechanisms of resistance. But it is not yet certain that they are more reliable in predicting patient response to therapy than standard cell line xenografts.

Most breast cancer PDXs grow at low efficiency in mice, and it is estimated that only 0–20% can actually engraft,

depending on the mouse model used, the tumor subtype, and whether the patient received therapy or not. The success rate in establishing PDX from hormonal receptor-positive tumors is particularly low. Given that a low success rate of engraftment requires the use of high numbers of immune-compromised mice, the application of PDX is both laborious and costly. Estrogen supplementation, the use of highly immunosuppressed mice (NSG), as well as co-injection of mesenchymal stem cells and/or Matrigel [68, 69] have increased the rates of engraftment, but they are still far below the 100% that would be needed for personalized medicine.

A possible explanation of such low engraftment rates is that not all murine growth factors and cytokines interact with their human cognate receptors [36]. Several strategies used to bypass this problem still only produce moderately low efficacies. These include co-injection of human breast cancer cells and partly irradiated human fibroblasts [70] or implantation of human breast cancer cells into mice carrying human bone grafts [71]. Perhaps one of the most promising strategies is to humanize mice by introducing human immune or hormonal components.

The three main mouse strains used for humanization studies are NSG, NOG, and the BRG (reviewed in [72]), which were generated by crossing the IL-2Rγ−/− and the SCID (NSG, NOG) or RAG2−/− (BRG) strains. These mice are defective in T, B, and NK cells, and their reduced macrophage and dendritic cell functions makes them suitable for humanization. In one of the first studies, human peripheral blood mononuclear cells (PBMCs) and tumor cells were co-transplanted into NSG mice. However, the tumor cells and PBMCs were derived from different donors; MHC mismatching led to an antitumor response or graft versus host disease [73]. Bypassing the rejection, human breast cancer cells were successfully implanted into mice injected with HLA-matched $CD34^+$ hematopoietic stem cells (HSC), which allowed an effective immune response [74, 75]. HSC transfer allows the development of most types of hematopoietic cells, including B and T lymphocytes, NK cells, monocytes, DC, erythrocytes, and platelets; their maturation and function can be further improved by engineering mice to express human cytokines such as hIL-2 [76], hIL-4, hIL-6 [37], HCSF/IL-3 [77], hIL-7 [78], or hTPO [79]. In the two recently generated mouse strains, MITRG and MISTRG, human versions of four genes encoding cytokines important for innate immune cell development were knocked into their respective mouse loci. The human cytokines support the development and function of monocytes, macrophages, and NK cells derived from human fetal liver or adult $CD34^+$ progenitor cells injected into the mice [80]. Overall, although there is still room for improvement; humanized mouse models are a promising strategy for efficient engraftment of patient-derived tumor biopsies and the study of their interaction with immune cell and hormonal components.

Although most of the steps in the metastatic cascade can be modeled, transplantation of breast cancer cells does not recapitulate the natural history of breast tumors, where loss of tumor suppressor genes and the expression of oncogenes transform normal cells. Furthermore, they do not allow study of cancer immune editing. Some of these deficiencies can be potentially recapitulated using genetically engineered mouse models (GEMMs), with mice fully engineered to express genetic lesions that result in autochthonous development of mammary cancer.

6.4 Genetically Engineered Mouse Models (GEMMs) of Breast Cancer

Early GEMMs of breast cancer were constructed by introducing mutations of interest into the mouse genome under the control of a mammary active promoter (e.g., MMTV). This method has several limitations, including the randomness of the insertion site and lack of control of gene copy number. Transgenes have also been integrated by specifically targeting the Rosa26 and H11 loci, where homozygous transgene expression does not disrupt endogenous coding sequences. Transgenes can also be inserted downstream of their endogenous promoters (knock-in), thus allowing physiological control of expression. Many of these early models expressed a particular oncogene or were deprived of a tumor suppressor in multiple cell types of the mammary gland.

6.4.1 Spatiotemporal Control of Transgene Expression

The mammary gland epithelium is composed of luminal cells that produce milk during lactation, together with surrounding myoepithelial/basal cells that have contractile activity and eject the milk [81]. Conditional mice have been developed that include the bacteriophage CRE-lox and the FLP/FRT system from *Saccharomyces cerevisiae*. Genetic lesions can be targeted to mammary cell types via expression of the Cre recombinase or flipase under the control of a specific promoter [82, 83]. Because several promoters are active during embryogenesis or at early phases of mammary gland development, constitutive genetic expression or deletion may impair normal development and preclude studies of the adult gland. Inducible systems such as tamoxifen-inducible Cre-ER [84] and tetracycline-controlled Cre expression [tTA (Tet-off) and rtTA (Tet-on) systems] [85, 86] have been used to induce mutations of interest at specific time points [87–90]. Because of their reversibility, tetracycline-inducible systems can be used to interrupt gene/oncogene expression after overt tumor development. This may reveal whether the tumor is addicted to the studied pathway [91, 92].

Commonly used promoters in GEMMs of breast cancer include the MMTV-LTR and the whey acidic protein (WAP) promoter, both highly active in mammary epithelial cells and responsive to hormonal stimulation. These promoters have been used extensively to drive mammary expression of several oncogenes, including neu/ErbB2, cyclin D1, cyclin E, *PIK3CA*, Ras, and Myc [22]. The MMTV-LTR is active in ductal and alveolar cells throughout mammary development (as early as 6 days postpartum) in a mosaic fashion, and its transcriptional activity increases during pregnancy [93]. A caveat of this model is the leakiness of MMTV-LTR in several organs, including hair follicles and salivary glands [22]. In contrast, the WAP promoter is restricted to the mammary gland, being active in 2–5% of cells during estrus and especially in most alveolar cells in midpregnant and lactating mammary glands [94, 95]. The WAP promoter has been used, for example, for targeted inactivation of tumor suppressors (e.g., pRb) [96] or expression of SV40 T-antigen [97] and *PIK3CA* mutants [89, 98].

Transgene expression can also be targeted preferentially to mammary basal or luminal cells. The results of in situ genetic lineage tracing studies suggest that cytokeratin 14 (K14) is expressed in mammary multipotent embryonic progenitors that give rise to basal and luminal lineages [88]. From birth onward, K14-positive cells become unipotent and give rise exclusively to basal cells [88]. This observation was later challenged by another lineage tracing study that identified bipotent K14-positive cells in pubertal and adult mammary glands [99]. K14-Cre or K14-CreER systems have been used to generate GEMMs, for example, to delete *Brca1* [100] *Tp53* [100] or express mutant *PIK3CA* [89]. The K5 promoter has also been used to target the basal population of the mammary gland [90]. Others have reported rare K5-positive cells that can be multipotent and that are present during puberty and pregnancy [99]. A model of stabilized β-catenin-induced mammary hyperplasia and carcinomas has been generated using K5 [101]. Expression of Lgr5, a downstream target of Wnt identified as a marker of adult stem cell populations in the small intestine, colon [102], stomach [103], and hair follicle [104], is expressed mostly in a subset of mammary basal cells in the nipple area [89, 105]. Expression of mutant *PIK3CA* in this subset of cells evoked mostly benign mammary tumors [89].

Lineage tracing results suggest that the K8 promoter is only active in the luminal compartment of the mammary gland, including luminal progenitor cells, and is involved in the maintenance of this compartment during puberty, adult life, and pregnancy [88]. Expression of the *PIK3CA*^{H1047R} mutant under K8 control led primarily to the formation of mammary tumors [89, 90].

Further promoters used to drive transgene expression in mouse mammary cells include the C3(1) [106], H19 [107], bovine β-lactoglobulin [108–110], and metallothionein promoters [111–114].

6.4.2 Modeling Genomic Alterations in GEMMs

GEMMs of breast cancer can be induced by the expression of an oncogene or the deletion of a tumor suppressor gene, mimicking either germline or somatic genetic defects.

6.4.2.1 Models of Human Germline Breast Tumor Mutations

The *BRCA1* [115, 116] and *BRCA2* [117, 118] germline heterozygous mutations were identified in the 1990s and have been defined as the highest risk factor for the development of familial breast and ovarian cancers [119]. Surprisingly, in contrast to the human situation, *Brca* heterozygous mice do not develop tumors, and homozygous deletion is embryonic lethal [95]. Several BRCA1 conditional models have been developed that exhibit mammary tumor formation in a subset of animals, albeit with a long latency [120]. Concomitant loss of *Tp53* considerably reduces tumor latency [121]. Brca1 tumors are typically ER and HER2/neu negative and resemble sporadic triple-negative basal-like breast cancers at the histopathological and molecular levels [8]. For a long time, this was taken as evidence for a basal origin of these cancers, but this was later challenged by data showing the accumulation of clonogenic aberrant *c-KIT*-positive luminal cells (luminal progenitors) in the breast tissue of mutant *BRCA1* carriers [122]. Moreover, inactivating *Brca1/Tp53* in luminal progenitors but not in basal cells of mice resulted in tumors closely resembling human BRCA1 mutant basal-like breast tumors [109], suggesting a luminal progenitor origin for basal breast cancer.

Brca2 inactivation driven by either WAP-Cre [123] or MMTV-Cre [124] was found to result in mammary carcinomas with a long latency. K14-Cre-mediated deletion produced no mammary tumors, except when combined with deletion of *Tp53* [87].

6.4.2.2 Modeling Somatic Alterations in Human Breast Cancer

Gene Amplification

The poster child model of gene amplification and overexpression and one of the earliest GEMMs of breast cancer is that of the gene encoding the receptor tyrosine kinase ErbB2 (neu/HER2); this member of the EGFR family is amplified in ~25% of human breast cancers [125–127]. HER2 overexpression defines a distinct histological and molecular subtype and is the target of the humanized monoclonal antibody trastuzumab [3, 4, 12]. Recently, cancer genome sequencing data have identified patients with breast tumors lacking *HER2* gene amplification but harboring somatic mutations in extracellular or kinase domains [13, 15, 128–130]. Some of these mutations have oncogenic potential in vitro and their

effect on sensitivity to anti-HER2 therapy [131] is being tested in clinical trials.

Mouse models of ErbB2-driven mammary tumorigenesis include, for example, the ErbB2/Neu-V664E (NeuNT: for Neu transforming) mutant driven by the MMTV-LTR [132, 133] and the endogenous *ErbB2* promoter [134]. While this particular mutation has never been found in human breast tumors, these models have been very useful for investigating several aspects of HER2-evoked tumorigenesis and signaling properties. In contrast to MMTV-LTR-driven expression, the expression of NeuNT under its endogenous promoter is in general not sufficient for the initiation of mammary carcinogenesis [134], highlighting a requirement for gene amplification and concomitant elevated protein expression. Expression of wild-type *ErbB2* under the control of MMTV-LTR also resulted in mammary tumors, albeit with a longer latency than NeuNT [135]. This suggests that additional genetic events are needed to transform the mammary epithelium. Notably, most tumors in these transgenic mice harbored in-frame small deletions in the transgene, and some have transforming capacity [136]. Mammary epithelium-specific expression of neu receptors harboring two of these in-frame neu deletions (NDL) (MMTV-neu-NDL mice) led to rapid induction of mammary tumors that frequently metastasized to the lungs [137]. Interestingly, the NDL mutant resembles an alternative splice form of the human *HER2* gene, Δ16HER2 [138]. The human spliced variant also induces the development of multifocal mammary tumors with a rapid onset [139] and confers tumorigenic and metastatic capacity to nonneoplastic MCF10A cells [140].

Further breast cancer mouse models of amplified genes include overexpression of cyclin D1 [141], cyclin E [142], Ras [143], Myc [143], and Wnt1 [144], mostly under the control of the MMTV promoter [22].

Gain of Function Mutations

The PI3K pathway is hyperactivated in over 70% of human breast cancers, due either to gain of function mutations in *PIK3CA* or *AKT1* or to loss of/reduced expression of PTEN, SHIP, or INPP4B; all these phosphatases dampen the activation of the pathway. *PIK3CA* mutations are present in 25–40% of all breast cancers, followed by about 8% with *AKT* mutations; each of these alterations occurs most frequently in ER-positive tumors [14, 130, 145, 146].

PIK3CA mutations or *AKT1* mutations seem to be an early event in breast tumorigenesis, as shown by the co-occurrence of these mutations in DCIS and invasive counterparts [147]. Several mouse models have been generated driven by inducible expression of the oncogene $PIK3CA^{H1047R\ or\ E545K}$ in different cells [148]. Using lineage tracing [149], recent studies have shed light on the source of tumor heterogeneity found in mutant $PIK3CA^{H1047R}$-induced mouse mammary tumors. Koren et al. [150] and Van Keymeulen et al. [90] showed that $PIK3CA^{H1047R}$ overcomes lineage restriction in adult mammary epithelial cells. Expression in luminal (K8 promoter) or basal (LGR5 or K5 promoters) cells resulted in their dedifferentiation into a multipotent stemlike state that gave rise to heterogeneous tumors expressing both basal and luminal lineage markers. Interestingly, the cell of origin dictated the phenotype of these tumors; tumors arising from basal cells usually displayed more benign histopathological characteristics, while those arising from luminal cells were more frequently malignant [89, 90].

With the publication of a comprehensive molecular characterization of breast cancer in 2012 [151], more than 30,000 genomic mutations were unraveled, and 35 of these were found to be frequently mutated. *PIK3CA*, *TP53*, and *GATA3* somatic mutations occurred at a frequency higher than 10% across all breast cancer subtypes; the next most frequent mutations were in *MAP3K1*, *PTEN*, *CDH1*, and *MAP2K4*. Some of these mutants have been used to produce GEMMs of breast cancer.

Other Models of Activated Pathways

The polyoma middle T (PyMT) antigen has also been expressed under the control of MMTV-LTR. Although PyMT is not a bona fide human oncogene, it induces several pathways that are hyperactive in human tumors, including ERK/MAPK, PI3K/AKT, and SRC. The PyMT is an extremely potent oncogene; within 4 weeks after birth, the mice develop mammary adenocarcinomas resembling human DCIS, both histologically and cytologically. Nonetheless, unlike human DCIS, most of these tumors lack ER expression. MMTV-PyMT mice develop metastases in the lungs as early as 6 weeks, which allows study of this multistep metastatic process [152].

Somatic Alterations in Tumor Suppressor Genes

In knockout/mutant models of tumor suppressor genes, mammary tumors often develop later than those caused by activation of oncogenes, and the mice usually manifest other cancers, especially of lymphatic and conjunctive tissues. Furthermore, these mammary tumors usually depend upon the activation of an oncogenic pathway; thus, a single tumor suppressor gene alteration can lead to different tumors depending on the collaborating oncogenic pathway.

The most commonly altered tumor suppressor gene in human breast cancer and the most commonly disrupted gene in GEMMs is *TP53*, which is mutated in about 30–40% of breast tumors, even higher in ER-negative subtypes [16, 153]. *Tp53* whole-body KO mice rarely develop mammary tumors (1–2% of all cancers), as most of the time, they succumb primarily to lymphomas [154]. This can be circumvented by transplantation of *Tp53* KO mammary glands into the cleared fat pad of *Tp53* WT syngeneic hosts [41] or the use of conditional knockouts [87]. The incidence of mammary tumors in p53-null animals is significantly higher in the

BALB/c murine background than in C57Bl/6×129, especially in p53 heterozygous mice [42]. Models of *Tp53* mutants R172H and R270H, the murine counterparts of human R175H and R273H hotspot mutants, have also been generated [155–157]. The spectrum of the developing tumors differs in these mice, and metastasis frequency is higher than for p53 KO or heterozygous animals [158]. A further model involving *Tp53* disruption is SV40 T-Ag [106], which induces transformation through inactivation of p53 and Rb family members. Remarkably, all of these models develop tumors with similar phenotypes, i.e., they are all heterogeneous with different grades, aneuploid, and genetically instable [158].

CDH1/E-cadherin is a tumor suppressor gene frequently downregulated in human lobular breast cancers [159]. Conditional loss of E-cadherin and p53 in mammary epithelial cells [160] accelerates the development of invasive and metastatic mammary carcinomas, presumably through induction of anoikis resistance and angiogenesis. The tumors closely resemble the human invasive lobular carcinoma (ILC); however, contrary to human ILC, the murine tumors are ER negative.

Germline *PTEN* mutation in Cowden disease predisposes to breast cancer [161]. Furthermore, sporadic breast cancers exhibit frequent loss of heterozygosity at the *PTEN* locus and reduced PTEN protein levels [151]. *Pten* deficiency accelerated mammary tumorigenesis and metastasis in an ErbB-2-driven mouse model [162] and mammary oncogenesis in MMTV-Wnt1 transgenic mice [163]. Full-body *Pten* KO leads to embryonic lethality, while heterozygous animals develop a broad range of tumors, including breast cancer [164]. Mammary epithelium-targeted deletion of *Pten* also led to the development of early-onset mammary tumors [165].

6.4.3 New Technologies for Generating GEMMs

Increasingly sophisticated techniques for manipulating the mouse genome and mouse embryos allow the generation of GEMMs with several mutations and offer unprecedented possibilities for validating and modeling the bona fide human drivers of tumorigenesis and metastasis identified by the sequencing of tumors [166]. An extensive review of the generation of GEMMs has been published elsewhere [167]. Until very recently, the interaction of different mutations in cancer was very difficult to test using mouse models, the crossing of different lines with specific mutations being laborious, costly, and time-consuming. With CRISPR-Cas9 technology, several different mutations can be introduced simultaneously into a given mouse in a relatively short time. This approach has been used successfully to generate GEMMs of lung cancer [168, 169], and its application to breast tumors is awaited.

6.4.4 Advantages and Disadvantages of GEMMs

In 1999, a group of experts in the mammary gland field, comprising medical and veterinary pathologists, gathered in Annapolis, Maryland, to systematize the classification of breast lesions arising from GEMMs in the framework of human disease. They evaluated 39 mouse models and proposed both a consensual nomenclature and guidelines for the classification of future models [170]. One of the main conclusions of this conference was that in general, genetically engineered mice give rise to tumors that do not resemble histologically the common types of human breast cancer (i.e., ER pathway-dependent tumors). A further limitation is the scarcity of breast cancer GEMMs that form metastases; when they do, the lung is the preferential organ of metastatic growth, while in humans, the bone, liver, lung, and brain are common metastatic sites. In humans, tumors spread initially via the lymphatic system and not via the blood, as in mice. Moreover, the mouse mammary stroma is rich in fat, while collagen and fibrous stroma are predominant in human breast. The latter may explain why mouse tumors exhibit much less fibrosis and inflammation than human breast tumors. Finally, although spatiotemporally controlled gene manipulation in mice is advantageous compared with initial GEMMs, the number of cells per gland targeted by the mutation largely overrides that found in sporadic human cancers. However, the benefits of GEMMs do outweigh their potential limitations. Many of the molecular lesions causing breast cancer in humans also lead to mammary tumors in mice. Furthermore, mammary tumorigenesis in mice is consistent with the multi-hit kinetics of human tumors. Another remarkable aspect is that certain GEMMs can recapitulate the level of heterogeneity that is found in human tumors. Accordingly, a recent genomic analysis of tumors from GEMMs of breast cancer revealed that individual mouse models could recapitulate the heterogeneity found among human breast cancer samples [171]. In addition, every subtype of human breast cancer was found to have its murine counterpart in terms of profile of signaling pathways activated. Notwithstanding important differences between the mouse and human immune systems [36], the presence of an intact immune system in GEMMs allows investigation of its involvement in autochthonous mammary cancer growth and progression, as well as the preclinical testing of immunotherapies. From a technical point of view, the mouse genome is fully sequenced and is easy to manipulate. Furthermore, mice have short generation times and ten mammary glands that provide material for ex vivo cell biological and biochemical studies.

Conclusions

Mouse models have made and will continue to make decisive contributions to our understanding of biological mechanisms of breast cancer initiation and progression,

to the preclinical validation of drug candidates, as well as to the discovery of new mechanisms of drug resistance and of predictive biomarkers. Technological advancements in genome editing, such as CRISPR-Cas9, the establishment of large PDX banks, and the development of humanized mouse models will continue to increase the number and diversity of available models.

The proper selection of a preclinical model is critical to drug discovery and should take into account the biological context of the target and the proposed mechanism of action of the drug [19]. Clearly, no one model will fit all drug discovery programs or recapitulate all aspects of the disease. As stated by B. Cardiff, a useful mouse model should be testable, predictive, and usable to falsify a hypothesis (Cardiff B, personal communication) [172, 173]. The model should provide a reproducible answer to a question that accurately verifies or rejects a hypothesis, whether it involves oncogene dependency, metastatic capacity, the validation of drug targets, or the identification of mechanisms of resistance. Careful attention must be paid to the attributes of the disease (molecular, biological, and structural) that are recapitulated in a specific model. The model should recapitulate a particular aspect of breast cancer biology and thus have relevant predictive power in human breast cancer.

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Box 1 General Considerations when Generating GEMMs (M. Barbacid, Keystone Symposium on Inflammation, Microenvironment and Cancer, 2008, Personal Communication)—Adapted from [174]

1. The genetic alteration studied should be found in human breast tumors.
2. The expression of the gene of interest should be targeted preferentially to the endogenous locus and not expressed as a transgene.
3. The genetic alteration should be introduced in a specific mammary cell subpopulation or lineage.
4. Expression or deletion should be inducible to allow activation/deletion in the adult gland or following tumor development.
5. The resulting tumors should recapitulate some features of human disease in terms of morphology, heterogeneity, pathways activated, and gene expression profile. In particular, more hormone-dependent (ER- and/or PR-positive) models should be developed.

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Part II

Epidemiology, Genetics and Prevention

Epidemiology, Lifestyle, and Environmental Factors

7

Patrick Maisonneuve

7.1 Global Patterns in Breast Cancer Incidence and Mortality

Estimated numbers of new breast cancer cases, cancer deaths, incidence, and mortality age-standardized rates by world regions and country for 2012 were compiled by the International Agency for Research on Cancer (IARC) and obtained from the GLOBOCAN platform. Detailed information about the source of data and the methods used for countries lacking incidence or mortality data are available in an article by Ferlay et al. [1].

Breast cancer is the most frequent cancer in the world among women with an estimated 1.7 million new cancer cases diagnosed in 2012, representing 25% of all cancers [2]. It is the most common cancer both in more developed and less developed regions of the world with slightly more cases in less developed (883,000 cases) than in more developed (789,000) regions. Incidence rates vary more than threefold across the world regions, with rates standardized according to the world population (ASR) ranging from 27 per 100,000 in Middle Africa and Eastern Asia to more than 90 in North America and Western Europe (Table 7.1). High incidence rates of breast cancer are also recorded in Australia and Southern Europe; intermediate rates in South America, in the Caribbean, and in Central and Eastern Europe; and low rates in most parts of Africa and Asia (Fig. 7.1).

Breast cancer is also the most frequent cause of death from cancer in women being responsible for 522,000 deaths, representing 15% of all cancers deaths in the world in 2012. It is the most frequent cause of cancer death in less developed regions (324,000 deaths, 14.3% of total), but it now ranks as the second cause of cancer death after lung cancer in more developed regions (198,000 deaths, 15.4%). Again, we observe a more than threefold range in age-standardized mortality rates across the world regions. Despite paucity of data from many of the less developed countries, highest mortality rates were estimated in Western Africa (20 per 100,000), while low mortality rates were estimated in Eastern Asia (6 per 100,000) (Table 7.1). Elevated mortality rates (>16 per 100,000) are observed in all European regions, intermediate rates (14–15 per 100,000) in North America and in Australia, and low rates (<14 per 100,000) in Central and South America (Fig. 7.1).

Because of the multifactorial aspects related to breast cancer incidence (see later section on lifestyle and hormonal risk factors) and breast cancer mortality (lowered by accessibility to mammographic screening and advanced treatment protocols) and of the wide variation of these factors between less developed and more developed countries, the ratio of mortality to incidence provides distinct information on the burden of breast cancer. In fact, we observe a wide variation in the mortality-to-incidence rates ratio worldwide mainly due to differences in survival between less developed and more developed regions. In most developed countries, the mortality-to-incidence rates ratio is lower than 0.20 (or one death for every five new cases), with lowest rates ratio observed in North America (0.16), in Australia and New Zealand (0.17), in Western and Northern Europe (0.18), and in Southern Europe (0.20). In contrast, highest mortality-to-incidence rates ratio are observed in Middle Africa (0.55), Western Africa (0.52), and Eastern Africa (0.51) with approximately one death for every two new breast cancer cases. Intermediate rates ratios are observed in South America (0.27) and Central and Eastern Europe (0.35) (Table 7.1).

Country-specific mortality data were also retrieved from the WHO mortality database for the most recent year for which they were available. The single country with the highest mortality rate from breast cancer is Armenia with 545 deaths reported in 2012, corresponding to an ASR of 24.6

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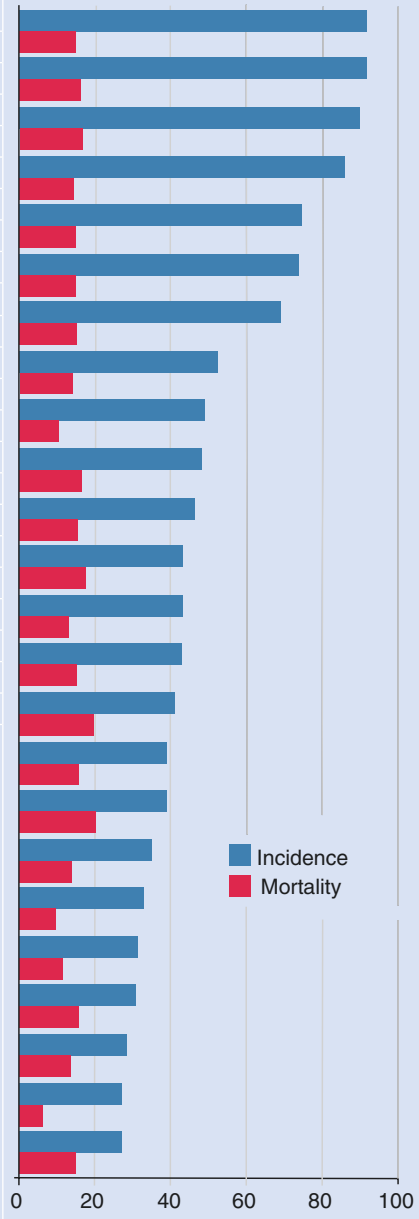
Table 7.1 Estimated breast cancer incidence and mortality by world area, 2012

		Incidence		Mortality		Rate
		Cases	ASR	Deaths	ASR	Ratio
Northern America		256,222	91.6	48,850	14.8	0.16
Western Europe		156,045	91.1	37,242	16.2	0.18
Northern Europe		78,249	89.4	17,915	16.4	0.18
Australia/New Zealand		17,550	85.8	3,620	14.5	0.17
Southern Europe		100,807	74.5	27,473	14.9	0.20
More developed regions		**788,200**	**73.4**	**197,618**	**14.9**	**0.20**
Polynesia		223	68.9	49	15.4	0.22
Southern America		115,881	52.1	32,014	14.0	0.27
Micronesia		128	48.8	27	10.4	0.21
Central and Eastern Europe		123,617	47.7	48,717	16.5	0.35
Caribbean		11,287	46.1	3,928	15.1	0.33
Northern Africa		39,512	43.2	15,577	17.4	0.40
World		**1,671,149**	**43.1**	**521,907**	**12.9**	**0.30**
Western Asia		42,485	42.8	14,810	15.1	0.35
Melanesia		1,376	41.0	633	19.7	0.48
Southern Africa		10,303	38.9	4,047	15.5	0.40
Western Africa		39,681	38.6	20,524	20.1	0.52
South-Eastern Asia		107,545	34.8	43,003	14.1	0.41
Central America		24,891	32.8	7,266	9.5	0.29
Less developed regions		**882,949**	**30.9**	**324,289**	**11.5**	**0.37**
Eastern Africa		33,472	30.4	17,028	15.6	0.51
South-central Asia		223,899	28.2	104,669	13.5	0.48
Eastern Asia		277,054	27.0	68,531	6.1	0.23
Middle Africa		10,922	26.8	5,984	14.8	0.55



Source GLOBOCAN 2012, IARC

per 100,000, followed by Barbados (24.0 per 100,000), Uruguay (18.9 per 100,000), Croatia (18.0 per 100,000), or Hungary (17.7 per 100,000). Many of the other countries with high mortality rates are situated in Northern, Central, and Eastern Europe and in South America. High rates are also reported for Israel (17.4 per 100,000). Many of the countries with the lowest mortality rates (<7 per 100,000) are situated in Central America (El Salvador, Guatemala, Nicaragua, Ecuador) and the west border of South America (Peru). Other countries with low mortality rates include the

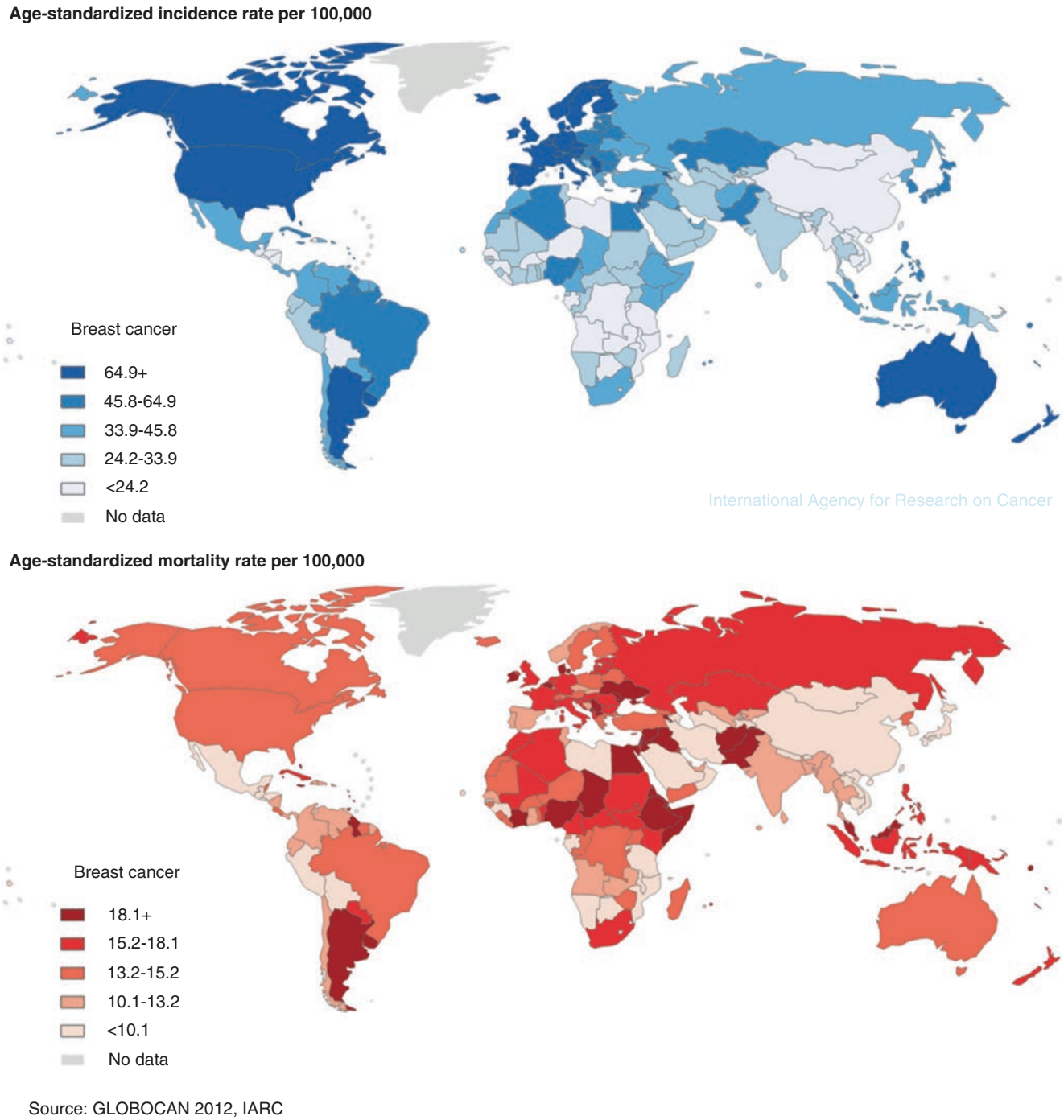


Fig. 7.1 Estimated age-standardized incidence and mortality rates from breast cancer, 2012. *Source* GLOBOCAN 2012, IARC

Republic of Korea (5.6 per 100,000) and Japan (9.0 per 100,000) (Table 7.2).

Trends in incidence of and mortality from female breast cancer are presented in Fig. 7.2 for selected countries with available long-term data. Between 1975 and 2010, breast cancer incidence rates rose by 20–50% in developed countries. This increase could largely be ascribed to change in reproductive patterns, the use of exogenous hormones, and

Table 7.2 Country-specific mortality from breast cancer in women (most recent available data)

Country	Year	Deaths	ASR	Country	Year	Deaths	ASR
Armenia	2012	545	24.6	Greece	2012	1990	14.0
Barbados	2012	63	24.0	Cuba	2013	1445	13.9
Uruguay	2013	634	18.9	Canada	2011	4958	13.8
Croatia	2013	994	18.0	Georgia	2014	515	13.8
Hungary	2013	2167	17.7	Cyprus	2012	102	13.6
Ireland	2012	689	17.7	USA	2013	40,860	13.4
Argentina	2013	5632	17.6	Switzerland	2013	1329	13.3
Denmark	2012	1123	17.4	Czech Republic	2013	1692	13.2
Israel	2013	1052	17.4	Finland	2013	866	13.1
Serbia	2013	1647	17.3	Belarus	2011	1184	13.0
Ukraine	2012	8076	17.2	Singapore	2014	411	12.8
Belgium	2012	2312	17.1	Sweden	2013	1473	12.8
Netherlands	2013	3161	16.7	Portugal	2013	1646	12.5
UK, Northern Ireland	2013	319	16.7	South Africa	2013	3033	12.0
Slovenia	2010	416	16.6	Costa Rica	2013	342	11.8
Russian Federation	2011	23,317	16.4	Norway	2013	631	11.8
UK, Scotland	2013	1013	16.4	Brazil	2013	14,204	11.7
Bulgaria	2012	1364	16.3	Paraguay	2013	324	11.7
Germany	2013	17,853	16.1	Spain	2013	6477	11.7
Latvia	2012	404	16.1	Kuwait	2013	82	11.6
New Zealand	2011	636	16.1	Belize	2013	11	11.5
UK	2013	11,476	15.9	Panama	2013	217	10.5
Estonia	2012	264	15.8	Turkmenistan	2013	252	10.5
Mauritius	2014	144	15.8	Chile	2013	1389	10.2
UK, England and Wales	2013	10,144	15.7	Suriname	2012	30	10.0
France	2011	11,557	15.6	Colombia	2012	2488	9.7
Slovakia	2014	898	15.5	Japan	2013	13,148	9.0
Luxembourg	2013	92	15.4	Mexico	2013	5337	9.0
Lithuania	2013	564	15.1	China, Hong Kong	2013	596	8.6
Malta	2014	75	15.0	Kyrgyzstan	2013	217	8.4
Romania	2012	3129	14.9	Dominican Republic	2012	356	7.4
Italy	2012	12,004	14.7	Ecuador	2013	518	6.8
Republic of Moldova	2013	477	14.6	Nicaragua	2013	170	6.8
Poland	2013	5816	14.4	Peru	2013	998	6.5
Venezuela	2012	2067	14.3	Egypt	2011	2093	6.0
Austria	2014	1535	14.1	Republic of Korea	2013	2231	5.6
Kazakhstan	2012	1415	14.1	Guatemala	2013	307	5.3
Australia	2011	2914	14.0	El Salvador	2012	168	5.1

Source World Health Organization, health statistics and information systems, mortality database (accessed on 30/11/2015)

intensification of breast cancer screening. This increase was particularly marked in Northern European countries, such as Denmark or Finland, and in England. Similar but somewhat slower increase in incidence was observed in Asian countries (China, Thailand, Japan, Singapore). Only modest increase in breast cancer incidence was observed in India or in the Philippines. In several countries including the USA, Canada, Australia, and New Zealand, the increase halted around year 2000, followed by a decline in incidence. This stabilization or decline in incidence could be due to the leveling of breast cancer screening in these countries [3].

In contrast to incidence trends, age-standardized death rates were stable in most countries until the mid-1980s to early 1990s and subsequently significantly decreased in the following period. However, in few countries from Asia (Japan, Republic of Korea, Singapore) or from Central America (Colombia, Costa Rica), breast cancer mortality rates are still increasing, but the absolute mortality rates in these countries remain among the lowest worldwide (around 10 per 100,000). More details about international variation in female breast cancer incidence and mortality are available in a recent article by DeSantis et al. [4].

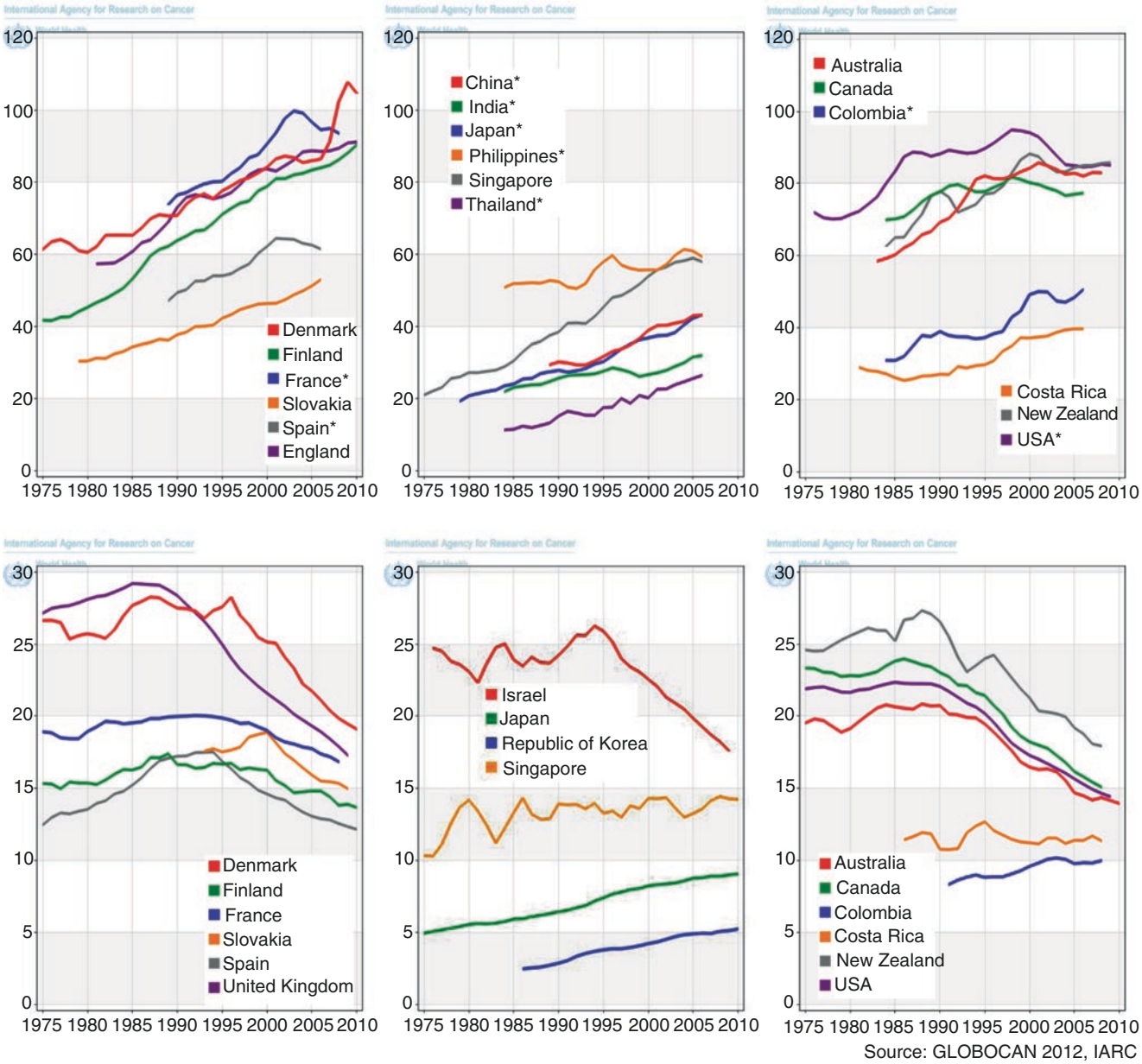


Fig. 7.2 Trends in incidence (*top*) of and mortality (*bottom*) from female breast cancer in selected countries: age-standardized rate (W) per 100,000. *Source* GLOBOCAN 2012, IARC

7.2 Demographic, Lifestyle, and Environmental Factors

7.2.1 Age- and Race-Specific Incidence Rate

As for most forms of cancer, breast cancer incidence increases with age. It represents a very rare form of cancer before 25 years of age to become a substantial form of cancer in women after age 50. Its incidence is strongly increasing until age 70 and declines at older age (Fig. 7.3). Overall, less than 5% of all breast cancer cases are diagnosed before age 40 and more than 60% after age 60. Breast cancer incidence also varies according to racial and ethnic groups. In the USA, incidence from breast cancer is higher among white than among black women. It is also lower among women of Hispanic or Asian origin and among Pacific Islanders.

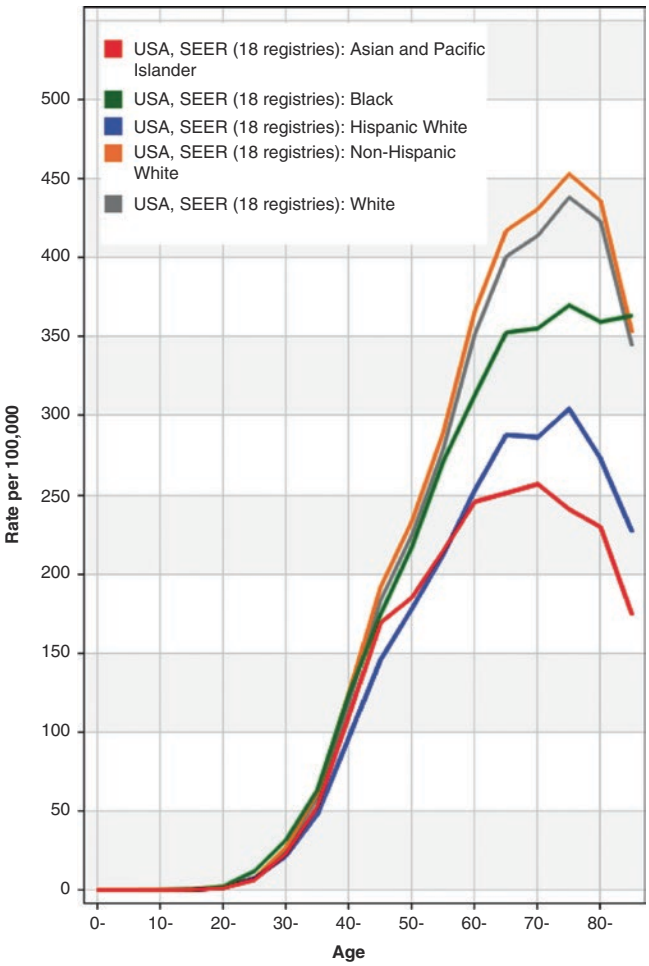


Fig. 7.3 Age- and race-specific incidence rates from female breast cancer in the USA (SEER 18 registries)

7.3 Reproductive and Hormonal Factors

Studies have shown that a woman's risk of developing breast cancer is related to her exposure to hormones that are produced by her ovaries (endogenous estrogen and progesterone). Reproductive factors that increase the duration and/or levels of exposure to ovarian hormones, which stimulate cell growth, have been associated with an increase in breast cancer risk. These factors include early age at menarche, late onset of menopause, later age at first pregnancy, and never having given birth.

In addition to direct effects on breast cells, pregnancy and breastfeeding both reduce a woman's lifetime number of menstrual cycles and thus her cumulative exposure to endogenous hormones.

7.3.1 Age at Menarche and Age at Menopause

Menarche and menopause are indicators of the onset and cessation of ovarian and related endocrine activity during women reproductive years. Early age at menarche and late age at menopause are known to increase women's risk of developing breast cancer.

A pooled analysis of individual data from 117 epidemiological studies shows that the risk of breast cancer decreases by 5% (95% CI 4.4–5.7%) for every 1-year delay in onset of menses [5], confirming results from an early study [6]. The same study shows that women breast cancer risk increases by 2.9% (95% CI 2.5–3.2%), for every year older at menopause. In addition, premenopausal women had a 43% excess risk (RR 1.43, 95% 1.33–1.52) of developing breast cancer than postmenopausal women of an identical age [5].

Breast cancer risk increased by a significantly greater factor for every year younger at menarche than for every year older at menopause, indicating that menarche and menopause may not affect breast cancer risk merely by extending women's total reproductive years [5].

7.3.2 Oophorectomy

Bilateral oophorectomy reduces breast cancer risk, likely because of reductions in levels of circulating ovarian hormones. In the Women's Contraceptive and Reproductive Experiences Study, bilateral oophorectomy was associated with reduced breast cancer risk overall (OR 0.59, 95% CI 0.50–0.69). In particular, women who had both ovaries removed before age 45 had about half the risk (ORs ranging from 0.31 to 0.52) of developing breast cancer compared to women who had a natural menopause, but not those who were older at surgery [7].

7.3.3 Pregnancy

There is well-established evidence that parity and early age at first full-term pregnancy are associated with reduction of breast cancer risk [8, 9]. The older a woman is when she has her first full-term pregnancy, the higher her risk of breast cancer. For example, women who have a first full-term pregnancy before age 20 have half the risk of developing breast cancer than that of women whose first full-term pregnancy occurs after the age of 30 [10]. The risk of breast cancer further declines with the number of full-term pregnancies even after adjustment for age at first birth [11]. However, a first childbirth

after age 30–35 no longer confers protection against breast cancer. Breast cancer risk is also transiently increased after a full-term pregnancy. Pregnancies that end as a spontaneous or induced abortion do not increase risk of breast cancer [12].

7.3.4 Breastfeeding

Breastfeeding is associated with a modest decrease risk of developing breast cancer, above and beyond that associated with multiple pregnancies. The longer women breastfeed, the more they are protected against breast cancer: the relative risk of breast cancer decreased by 4.3% (2.9–5.8) for every 12 months of breastfeeding in addition to a decrease of 7.0% (5.0–9.0) for each birth [13].

7.3.5 Oral Contraceptives

Oral contraceptive use is associated with a slight and transient increased risk of breast cancer. Authors of a meta-analysis based on individual data from 54 studies estimated a relative risk of 1.24 (95% CI 1.15–1.33) for current users. This increased risk declines to 1.16, 1–4 years after stopping oral contraceptive use, and to 1.07, 5–9 years after, while no risk was seen after 10 years from cessation [14].

Several hypotheses have been made about the influence of oral contraceptives on breast cancer risk. It was thought to be more important before cellular differentiation occurring with a full-term pregnancy and possibly to vary according to the type or formulation used. A large nested case–control study suggested that recent use of contemporary oral contraceptives was associated with an increased breast cancer risk, which may however vary by formulation [15]. In this study, the authors did not find an association with low-dose estrogen oral contraceptives. In another recent study from South Africa, a significant increased risk of breast cancer was found for women using injectable progestin-only contraceptives, but again the risk decreased to normal few years after cessation [16].

7.3.6 Hormone Replacement Therapy

There is a large body of evidence that long-term use of combined hormone replacement therapy (HRT) containing estrogen plus progestogen given to relieve the climacteric symptoms of menopause is associated with an increased risk of breast cancer [17, 18]. The increased risk is greater for women starting HRT soon after the onset of menopause [19] with linear diminishing influence as time from menopause increased [20]. The risk also dissipates within 2–5 years of discontinuation of HRT use, regardless of the duration of treatment. In a meta-analysis, use of combined estrogen–progestogen therapy was associated with a 7.6% increase in breast cancer risk per year of use [21].

Contrasting results observed for women receiving estrogen-only therapy in the Women's Health Initiative (WHI) trials in the USA [22] and the Million Women Study in the UK [23] could be largely ascribed to differences in age distribution and anthropometric measures [24].

7.4 Other Demographic and Lifestyle Factors

7.4.1 Height

Increasing height, which is influenced by childhood nutrition, genetic predisposition, prenatal exposures, and IGF levels, has been associated with an increase of breast cancer, particularly in postmenopausal women. In a pooled analysis of individual data from prospective cohort studies, van den Brandt et al. estimated that the risk of breast cancer increases by 7 percent for 5 cm of height increment (RR 1.07; 95% CI 1.03–1.12) in postmenopausal women [25].

Length at birth and during childhood have also been positively associated with breast cancer risk suggesting that factors influencing fetal, childhood, and adolescent growth are important independent risk factors for breast cancer in adulthood. In a cohort of 16,016 women in Norway, birth length was positively associated with risk. Women who were ≥53 cm had a relative risk of 1.8 (95% CI 1.2–2.6) compared with women who were shorter than 50 cm, after adjustment for multiple confounding factors [26]. A 5 cm increase in height at age 8 was associated with an 11% increased risk of breast cancer (RR 1.11; 95% CI 1.07–1.15). A 5 cm height increase between age 8 and 14 was associated with a 17% increased risk of breast cancer (RR 1.17; 95% CI 1.09–1.25). Compared to girls measuring around 151 cm at age 14, those measuring 168 cm had a 50% increased risk of developing breast cancer during adulthood (RR 1.51, 95% CI 1.36–1.68) [27].

7.4.2 Obesity

There is strong evidence that adiposity is associated with breast cancer risk, but this association varies by menopausal status. Elevated body mass index (BMI) is associated with an

increased risk of breast cancer in postmenopausal women, and there is growing evidence that obesity is associated with poor prognosis in women diagnosed with early-stage breast cancer. In a meta-analysis of 31 observational studies comprising 23,909 breast cancer cases, an increase of BMI of 5 kg/m^2 has been associated with a 12% increased risk of cancer (RR 1.12; 95% CI 1.08–1.16) in postmenopausal women [28].

On the contrary, BMI increase was inversely associated with the risk of premenopausal breast cancer (RR 0.95 for 5 kg/m^2 increase; 95% CI 0.94, 0.97), but this association varies by ethnicity, remaining significant only among Africans and Caucasian women [29].

In an analysis of individual data from eight prospective studies, the Endogenous Hormones and Breast Cancer Collaborative Group concluded that the increase in breast cancer risk with increasing BMI among postmenopausal women is largely the result of the associated increase in estrogens, particularly bioavailable estradiol [30].

7.4.3 Diet

There was almost universal agreement that diet or nutritional practices in some form must play a role in establishing breast cancer risk. This was a credible assumption to explain the remarkable changes that occur in breast cancer risk following migration from low-risk to high-risk areas of the world. However, no specific component of the adult diet and no particular nutrient have been consistently associated with breast cancer risk [31].

The results of a large meta-analysis of 26 published studies from 1982 to 1997 [32] and of a pooled analysis of 8 cohort studies [33] suggest that fruit and vegetable consumption during adulthood is not significantly associated with reduced breast cancer risk.

A pooled analysis of individual data from seven prospective studies in four countries comprising 337,819 women and 4980 breast cancers also suggested a lack of association between total fat, saturated fat, mono- and polyunsaturated fat intake and breast cancer risk [34].

Based on an extensive review of the literature, an experts panel for the World Cancer Research Fund classified as "Limited evidence—no conclusion" the association with dietary fiber, vegetables and fruits, soya and soya products, meat, fish, milk and dairy products, folate, vitamin D, calcium, selenium glycemic index, dietary patterns, and breast cancer. The expert panel found "Limited—suggestive" association for total fat and postmenopausal but not premenopausal breast cancer [31].

7.4.4 Physical Activity

There is more convincing information for an inverse association between physical activity and breast cancer risk [35]. The evidence is stronger for postmenopausal breast cancer, with risk reductions ranging from 20 to 80%, than for premenopausal breast cancer. Moderate physical activity during adolescence or young adulthood has also been associated with a lowered risk of developing breast cancer. Lagerros et al. reported that each hour increase of recreational physical activity/week during adolescence (in 12–24-year-old females) was associated with a 3% (95% CI 0–6%) risk reduction of breast cancer [36].

According to the World Cancer Research Fund, physical activity probably protects against breast cancer postmenopause, and there is limited evidence suggesting that it protects against this cancer diagnosed premenopause [31].

7.4.5 Alcohol and Tobacco

There is substantial evidence that alcohol consumption increases breast cancer risk. A collaborative reanalysis of data from 53 epidemiological studies [37] demonstrated that compared to women who abstained to drink alcohol, those who consumed 35–44 g of alcohol daily (corresponding to 3–4 alcoholic drinks per day) had a 30% increased risk of developing breast cancer (RR 1.32; 95% CI 1.19–1.45). The risk increases by nearly 50% for women who drink an equivalent of 45 g of alcohol per day (RR 1.46; 95% CI 1.33–1.61). It is estimated that the relative risk of breast cancer increased by 7.1% (5.5–8.7) for each additional 10 g per day intake of alcohol, i.e., for each extra unit or drink of alcohol consumed on a daily basis.

In the same report, the authors found no evidence of an association between smoking and breast cancer risk, after adjustment for alcohol drinking. Despite mixed result in earlier studies, there is growing evidence that smoking may slightly increase the risk of breast cancer. In a recent meta-analysis, current (HR 1.12; 95% CI 1.08–1.16) and former smoking (HR 1.09; 95% CI 1.04–1.15) were weakly associated with breast cancer risk; a stronger association (HR 1.21; 95% CI 1.14–1.28) was observed in women who initiated smoking before first birth [38].

7.4.6 Radiations

Observations in Hiroshima and Nagasaki atomic bomb survivors [39] and in women who have received therapeutic radiation treatment to the chest [40] document increased

breast cancer risk. It is postulated that exposure during adolescence, a period of active breast development, enhances the effect of radiation exposure [41].

7.4.7 Occupational Exposure

A recent an exhaustive review of environmental and occupational causes of cancer, considered "Suspected" evidence of a causal link when results of epidemiological studies are mixed, yet positive findings from well-designed and conducted studies warrant precautionary action and additional scientific investigation, while "Strong" causal evidence of a causal link was based primarily on a Group 1 designation by the International Agency for Research on Cancer. For all occupational exposures evaluated (pesticides, polycyclic aromatic hydrocarbons (PAHs), ethylene oxide, and polychlorinated biphenyls (PCBs)), there was only a suspected association with breast cancer. Of interest, a pooled analysis of data from five large studies in the USA does not support an association of breast cancer risk with plasma/serum concentrations of 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE) or PCBs [42].

7.5 Genetic Factors

Up to 10% of breast cancer cases in westernized countries are due to genetic predisposition. Having a first-degree relative with breast cancer approximately doubles the risk of developing breast cancer. The risk varies with the age at which the affected relative was diagnosed and with the number of affected relatives. A first-degree relative with ovarian cancer also confers a modest risk of breast cancer. This will be extensively detailed in the next chapter.

Conclusion

Breast cancer is the most frequent form of cancer and the leading cause of cancer death among women worldwide. High age-standardized rates are observed in North America, in Australia and New Zealand, and in Northern and Western Europe; intermediate in Central and Eastern Europe, South America, and the Caribbean; and low in most of Africa and Asia. International variations in breast cancer incidence and mortality rates reflect differences in risk factors, in access and dissemination of breast cancer screening and modern treatment protocols.

Nongenetic breast cancer risk factors include consuming alcohol; not having children or having children at late age; not breastfeeding; using or having recently used oral contraceptives or hormone replacement therapy after menopause; being tall, overweight, or obese; not being physically active; or having been irradiated to the chest.

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Breast Cancer Genetics

8

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Abbreviations

Endo BX	Endometrial biopsy
HBOC	Hereditary breast and ovarian cancer
HDGC	Hereditary diffuse gastric cancer
MMG	Mammography
MRI	Magnetic resonance imaging
RRSO	Risk-reducing salpingo-oophorectomy
TVUS	Transvaginal ultrasound

8.1 Introduction

Breast cancer is the most common form of cancer and the second most common cause of cancer death among women in the developed world. It has been estimated that women in the USA have a 12% lifetime risk of developing breast cancer beginning in their 20s, with a risk of developing cancer in the next 10 years for a woman in her 30s of approximately one in 250, and 1 in 50 by age 50 [1]. In the modern era, the goal is to identify women at increased risk to try to prevent their breast cancers. Currently, it is well known that individual risk for breast cancer is increased in individuals carrying a mutation in a predisposing gene and in others with a number of affected relatives with early age of disease onset in whom no specific mutation has been identified [2]. Approximately 5–10% of the total breast cancer cases follow a Mendelian inheritance pattern (autosomal dominant) and are characterized as hereditary. An additional 15–20% of breast cancer cases are named familial, referring to women who have two or more first- or second-degree relatives with the disease, without an identified gene. Among hereditary breast cancer cases, at least 30% are caused by germline mutations in the high-penetrance genes *BRCA1* and *BRCA2* [2], and the risk associated with less prevalent and more moderately penetrant genes is the subject of intense research effort.

Knowledge of somatic genetics and genomics has increasingly broad implications in oncology, not only in the identification of new treatments such as trastuzumab for HER2-positive breast cancer [3] but also as the basis for assays evaluating recurrence risk and treatment guidance, such as Oncotype DX, MammaPrint, and PAM50 [4, 5]. The germline or heritable genome provides important implications for the identification of high-risk individuals, ultimately for the development of effective cancer prevention strategies across the tumor types for which the genes confer increased cancer risk. However, there have been implications for therapeutic interventions as well. Prominent examples of the latter include the data for cis- and carboplatins in *BRCA1/2*-associated breast and ovarian cancers [6, 7] and PARP inhibitors in *BRCA1/2*-associated breast, ovarian, pancreatic, and prostate cancers. For this chapter, the ultimate goal of germline information is to identify individuals and families not yet affected, but at high risk of developing tumors who might be interested in preventive interventions that might effectively reduce their cancer mortality, at least for those cancers for which they have greatest risk.

The evolution of next-generation sequencing technologies has enabled parallel simultaneous testing of multiple genes beyond *BRCA1/2*, leading to concurrent analysis of breast cancer predisposition genes with a range of associated cancer risks, including high- and intermediate-/moderate-penetrance genes. The efficiency of next-generation sequencing has also increased the speed of the analysis, thereby reducing turnaround time, and has significantly reduced the costs. The effect of the widespread introduction of NGS technologies, therefore, has been to increase access to more comprehensive genetic analyses. However, access to this technology has also brought new challenges: the identification of the ideal candidates for utilization of panels, the

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appropriate management of patients with mutations in genes whose penetrance still is not clear, the increase in the number of variants of uncertain significance (VUS) found, and the incidental finding of mutations in genes in families that do not have a clear phenotype of that syndrome.

8.2 Genetic Testing

Patients with breast cancer should be offered genetic testing, according to consensus guidelines. The guidelines may differ in some specifics depending on the country, but most would concur that individuals with breast cancer should be tested if they are diagnosed at a young age (< age 50 is a frequent criterion), when they present with triple-negative histology, or with ovarian cancer, and recently castrate-resistant prostate cancer or pancreatic cancer. Individuals without cancer are often eligible for genetic testing based on family cancer history that includes the above tumors in various configurations.

The ESMO guidelines comprise widely accepted clinical criteria for referral for genetic evaluation of unaffected individuals with family histories as follows: three or more breast and/or ovarian cancer cases, at least one <50 years, two breast cancer cases <40 years, male breast cancer and ovarian cancer or early-onset female breast cancer, Ashkenazi Jewish individuals with breast cancer of <60 years, young onset bilateral breast cancer, and breast and ovarian cancer in the same patient. In some countries, the criterion for testing is based on an a priori 10–20% probability of finding a mutation based on predictive models such as BRCAPRO, BOADICEA, or Manchester score [8]. However, others believe these criteria are too strict [9].

Much of the early data came from studies of *BRCA1* and *BRCA2*, and still the most substantial and stable data come from the study of individuals with mutations in these genes. They were the first identified, and data come from large cohorts, including the CIMBA consortium, which is custodian for tens of thousands of *BRCA1/2* mutation carriers that have been systematically studied for more than 10 years [10]. For these patients the most well-established genes to be evaluated are *BRCA1/2*, especially because those are the most common genes involved in breast cancer susceptibility and also because those are the ones for which we have the best data regarding penetrance and management.

At this time, however, the availability of multigene panel testing has raised new issues regarding eligibility for gene testing beyond BRCA and new challenges about interpretation and management of the results. According to the National Comprehensive Cancer Network (NCCN) in the USA, patients who have a personal and family history suggestive of a specific syndrome may be best evaluated by a target gene analysis. For those whose history can be explained by more than one gene—which is the majority of patients—evaluation by panel can be more efficient and/or cost-effective. For those patients with *BRCA1/2*-negative tests and with a strong family history, panels can be a good option in increasing the chance of finding a mutation in another predisposing gene by about 4% [11].

In this chapter we will first present the most important genes related to breast cancer risk, detailing their prevalence, associations with different cancers, and any pathologic characterizations and/or molecular features of those cancers. We will then discuss the clinical management of individuals carrying significant alterations in each gene as regards surveillance, risk-reducing surgery, and other available treatment regimes.

8.3 High-Penetrance Genes

8.3.1 *BRCA1* and *BRCA2*: Hereditary Breast and Ovarian Cancer (HBOC) Syndrome

The first gene associated with hereditary breast cancer is *BRCA1*, located on chromosome 17q. This gene was identified in 1990 using linkage analysis in families with suggestive pedigrees [12]. In 1994, *BRCA2* was mapped to chromosome 13q, and together they became the most important and studied genes related to hereditary breast and ovarian cancers [13].

Female carriers of pathogenic variants (mutations) in *BRCA1* or *BRCA2* have a lifetime risk of breast cancer of 50–85% [14–16]. In addition, there is a substantially increased risk of ovarian cancer, with an estimated lifetime risk of 20–60% for *BRCA1* carriers and 10–20% for *BRCA2* carriers [14, 17]. There are other tumors associated with mutations in BRCA2 in particular, and cases of melanoma, prostate, and pancreatic cancer [18] should be taken into account when considering family history.

When considering histopathological features, it is well established that *BRCA1*-related breast tumors, as a group, differ from non-*BRCA1* tumors in terms of histological phenotype. Malignant primary breast tumors of *BRCA1* mutation carriers are more likely to be high grade with medullary subtype features, including greatly increased mitotic count, pushing margins, lymphocytic infiltrate, trabecular growth pattern, and necrosis. Most importantly, about 70% do not express estrogen or progesterone receptor or HER2 (triple-negative breast cancer—TNBC) [19], but perhaps 20% are positive for ER and PR, and the remaining 5–10% are HER2 positive [20, 21]. This distribution has led to recognition that a significant subset of TNBC will occur in women who carry a germline *BRCA1* mutation, even in the absence of family cancer history, and has made TNBC in women younger than age 60 at diagnosis, a criterion for BRCA testing.

The majority of *BRCA2*-associated tumors are invasive ductal, high-grade, estrogen and progesterone receptor positive and negative for HER2. They are less likely than controls to express the basal cytokeratin CK5 or to overexpress HER2/neu protein. In fact *BRCA2* tumors are predominantly high-grade invasive ductal carcinomas of no special type, and they demonstrate a luminal phenotype despite their high histologic grade [20, 22, 23].

Among hereditary breast cancer cases, at least 30% are attributed to germline mutations in the high-penetration genes *BRCA1* and *BRCA2*, but these numbers can vary across different populations due to founder effects [2, 24].

Evidence shows that, in addition to the presence of a mutation on *BRCA1/2*, other factors such as environment, lifestyle factors, mutation locations, and the presence of some SNPs might be important to precisely estimate the quantitative cancer risks associated with specific BRCA mutations in carriers [25] and may affect the clinical management of these patients in the future. Direct evidence for genetic modifiers of risk has been provided through studies that investigated the effects of common breast and ovarian cancer susceptibility variants on cancer risk for *BRCA1* and *BRCA2* mutation carriers, identified through genome-wide association studies (GWAS) or candidate gene studies in the general population [26–30]. The GWAS data required independent validation but could provide helpful stratification of risk to assist women with the planning of risk-reducing measures, childbirth, and other aspects of life. Another important issue, addressed in a large analysis of genotype/phenotype data published by Rebbeck et al. on behalf of the CIMBA consortium, is that breast and ovarian cancer risks vary with the precise location of the mutation in *BRCA1* or *BRCA2*. The clustering of mutations in the large exon comprising the "ovarian cancer cluster region" (OCCR) and other associations with breast cancer cluster regions (BCCR), for both *BRCA1* and *2,* speak to the challenge of genetic heterogeneity [31].

8.3.2 *TP53*: Li–Fraumeni Syndrome

The *TP53* germline mutations give rise to Li–Fraumeni syndrome (LFS), a rare inherited cancer predisposition syndrome associated with approximately 1% of breast cancer cases. Germline mutations in this gene predispose to a wide spectrum of malignancies, including sarcomas, brain tumors, adrenocortical carcinomas, and leukemias, occurring at any point in an individual's lifetime, with a median age at diagnosis of first malignancy of 25 [32]. Otherwise, somatic *TP53* mutations are the most common mutations in adult adenocarcinomas.

TP53 is a tumor suppressor gene located on chromosome 17p13.1 that plays a major role in the regulation of cell growth [33]. Approximately 70% of patients with classic LFS criteria have a detectable TP53 germline mutation [34]. Mutations are most commonly missense, but deletions of the coding or promoter region of p53 can also occur [35].

TP53 mutation carriers face a lifetime risk of cancer that exceeds 90% [36]. Breast cancer is the most frequent malignancy among female *TP53* mutation carriers and represents up to one third of all cancers in LFS families [37]. Overall, although LFS is responsible for a small fraction of breast cancer cases, a woman with LFS has a breast cancer risk of 56% by the age of 45 and greater than 90% by the age of 60, which accounts for a 60-fold increased risk for early-onset breast cancer when compared to the general population [38, 39]. Women with LFS-related breast cancer have a tendency to present at a very young age (20s or 30s) with a more advanced disease (tumor > 5 cm, positive axillary nodes) [40–42]. Furthermore, recent studies have shown that two thirds of LFS-associated breast cancer tumors are positive for epidermal growth factor receptor 2 (HER2/neu) and/or estrogen and progesterone receptor [41, 42]. It is possible that the outcome of LFS patients identified in the modern era will be better because of the introduction of therapies that effectively target HER2.

Recently, with clinical availability of NGS-based multigene panel tests that analyze dozens of hereditary cancer genes in parallel usually including TP53, new challenges arise due to many patients without criteria for LFS being tested [43]. This less strict approach to genetic evaluation has resulted in the identification of mutations in various established hereditary cancer genes in patients who lack the expected phenotype, raising important questions about prevalence, penetrance, and phenotypic spectrum [44–46]. This technology has also enabled the identification of low-level DNA variation consistent with germline mosaicism or somatic interference, which can be particularly challenging in clinical practice [47].

8.3.3 *CDH1*: Hereditary Diffuse Gastric Cancer Syndrome

E-Cadherin germline mutations are responsible for the development of hereditary diffuse gastric cancer (HDGC), an autosomal inherited syndrome [48]. These constitutional alterations were first identified in a Maori population with a remarkable clustering of diffuse gastric cancer in a single large kindred [49]. This large pedigree was characterized by the presence of multiple gastric tumors as well as lobular breast cancers (LBCs) among female family members. A germline mutation in *CDH1* was identified among affected relatives. The *CDH1* gene is located on chromosome 16q22.1 and encodes the E-cadherin protein [50], which is critical for establishing and maintaining polarized and differentiated

epithelia through intercellular adhesion complexes, functioning as a cell invasion suppressor. Aberrant E-cadherin activity leads to loss of cell adhesion, increased cell motility, and metastatic ability of the tumor [51, 52].

The penetrance of gastric cancer in people with *CDH1* mutations is reported to be 70% for men and 56% for women by age 80. Furthermore, the cumulative risk of LBC for women with a *CDH1* mutation is estimated to be 42% by age 80. There is currently no evidence that the risk of other cancer types in individuals with a *CDH1* mutation is significantly increased [53].

Apart from the well-documented association between LBC and HDGC syndrome, novel E-cadherin germline mutations have recently been detected in individuals without history of HDGC. Recent studies have provided evidence that early-onset LBC might be the first manifestation of HDGC. Benusiglio et al. [54] identified E-cadherin germline deleterious mutations in three bilateral LBC cases (age at onset >50 years) not fulfilling the International Gastric Cancer Consortium criteria, negative at the beginning for HDGC in first- and second-degree relatives and without *BRCA1* and *BRCA2* alterations. Interestingly enough, E-cadherin mutations have been identified in four bilateral early-onset LBCs (age at onset >50 years) with no family history of HDGC [55].

Recently the International Gastric Cancer Consortium has added a novel criterion, recommending genetic testing also in bilateral LBC patients or women with a family history of two or more cases of LBC (>50 years at onset) [53]. However, *CDH1* germline mutations have also been identified in isolated cases with age at onset >45 years [56].

In a recent study, penetrance data for *CDH1* mutation carriers has been updated based on affected individuals who presented clinically with HDGC or LBC, from 75 families with pathogenic *CDH1* mutations. The cumulative risk of HDGC for *CDH1* mutation carriers by age 80 is reported to be 70% for men (95% CI 59–80%) and 56% for women (95% CI 44–69%). The cumulative risk of LBC for women with a *CDH1* mutation is estimated to be 42% (95% CI 23–68%) by age 80. There is currently no evidence that the risk of other cancer types in individuals with a *CDH1* mutation is significantly increased [57].

8.3.4 *PTEN*: Cowden Syndrome

Germline mutations in *PTEN* are the cause of Cowden syndrome (CS) or *PTEN* hamartoma tumor syndrome (PHTS). Hamartoma is a benign, focal malformation that resembles a neoplasm in the tissue of its origin. This is not a malignant tumor, and it grows at the same rate as the surrounding tissues. It is composed of tissue elements normally found at that site, but growing in a disorganized mass.

CS is an autosomal dominant multisystem disorder characterized by increased risks of malignant and benign tumors of the breast, thyroid, endometrium, and other organs, as well as a combination of mucocutaneous findings such as trichilemmomas, oral papillomas, and acral keratoses [58]. PHTS can be differentiated from other hereditary cancer syndromes based on personal as well as family history. However, many of the benign features of CS are common in the general population, making the diagnosis of CS challenging [59].

More than 90% of CS individuals with germline (heritable) *PTEN* mutations are believed to manifest some feature of the syndrome, although rarely cancer, by age 20, and by age 30, nearly 100% of mutation carriers are believed to have developed at least some of the mucocutaneous signs. CS remains underdiagnosed because of its variable expression (often with only subtle skin signs); consequently, the current prevalence estimate of one in 200,000 is still likely to be an underestimate [60].

PTEN is a phosphatase and tensin homolog located on chromosome 10q23.3 with phosphatidylinositol-3-kinase (PI3K) phosphatase activity. *PTEN*'s precise function is not clear; however, dysfunctional *PTEN* leads to the inability to activate cell cycle arrest and apoptosis, leading to abnormal cell survival [61]. Approximately 80% of affected individuals will have a detectable *PTEN* mutation that may include a missense, point, deletion, insertion, frame shift, or nonsense mutation [62]. Among the 20% of patients with no identifiable *PTEN* mutation, half may bear a mutation in *PTEN* promoter [63].

What is histologically unique in patients with CS is ductal adenocarcinoma surrounded by hyalinized collagen, and this suggests a diagnosis of CS. Women with CS also have a high risk (67%) of benign breast disease, such as fibroadenomas, microcysts, adenosis, and apocrine metaplasia. Mammary hamartomas are characteristic of this group of patients and might be multiple and bilateral. Colocalization with breast cancer is frequent [64]. In patients with germline *PTEN* mutations and thus PHTS, three studies to date have examined risks for malignancy [65–67]. The largest, by Tan et al., identified greatly increased lifetime risks for breast, thyroid, renal, and endometrial cancers and slightly elevated risks for colorectal cancers and melanoma [65].

Early estimates of breast cancer risk for females with *PTEN* mutations were traditionally reported to be around 25–50% [68, 69]. More recent studies have reexamined the lifetime risks for malignancy in patients with germline *PTEN* mutations and have found that early risk figures may have been underestimates [65–67, 70]. The largest of the three studies by Tan et al. identified increased risks for several types of cancer, with the highest risk estimate increase for female breast cancer. Tan et al. [68] identified an 85%

lifetime risk, beginning around age 30, for female breast cancer with 50% penetrance by age 50. This risk figure is comparable to that quoted for patients with HBOC syndrome [67] but has been controversial.

According to NCCN guidelines, the presence of a known *PTEN* mutation in an individual's family is a clear indication for genetic testing for CS. Genetic testing for CS is also warranted when several diagnostic criteria are met (Table 8.1) [71], which are mainly based on clinical phenotype and the development of neoplasia. The *PTEN* risk calculator was developed by the team at the Cleveland Clinic to evaluate patients with suspected CS and is available on their website. This tool was developed from a multicenter prospective study in which 3042 probands satisfying relaxed CS clinical criteria were accrued, and it can help to distinguish patients more likely have clinical CS and test positive for PTEN mutations [68]. This tool was also proven to be cost-effective and provided a well-calibrated estimation of pretest probability of *PTEN* status [60].

Table 8.1 National comprehensive cancer network 2015 Cowden syndrome criteria [71]

Major criteria
Breast cancer
Endometrial cancer (epithelial)
Thyroid cancer (follicular)
Gastrointestinal hamartomas (including ganglioneuromas but excluding hyperplastic polyps)
Lhermitte–Duclos disease (adult)
Macrocephaly (97th percentile: 58 cm for adult women, 60 cm for adult men)
Macular pigmentation of the glans penis
Multiple mucocutaneous lesions (any of the following):
Multiple trihilemmomas (3, at least 1 proven by biopsy)
Acral keratoses (3 palmoplantar keratotic pits and/or acral hyperkeratotic papules)
Mucocutaneous neuromas (3)
Oral papillomas (particularly on the tongue and gingival), multiple OR biopsy-proven OR dermatologist diagnosed
Minor criteria
Autism spectrum disorder
Colon cancer
Esophageal glycogenic acanthosis
Lipomas
Intellectual disability (i.e., intelligence quotient/75)
Renal cell carcinoma
Testicular lipomatosis
Thyroid cancer (papillary or follicular variant of papillary)
Thyroid structural lesions (e.g., adenoma, multinodular goiter)
Vascular anomalies (including multiple intracranial developmental venous anomalies)
Three or more major criteria, but one most include Lhermitte–Duclos disease, macrocephaly or GI hamartoma
Two major criteria plus three minor criteria

8.3.5 *STK11*: Peutz–Jeghers Syndrome

Germline mutations in the *STK-11* gene are the cause of Peutz–Jeghers syndrome (PJS). PJS is a rare autosomal dominant disorder characterized by multiple gastrointestinal hamartomatous polyps and mucocutaneous pigmentations of the lips, buccal mucosa, and digits. These lesions fade during puberty, with the exception of those in buccal mucosa. Polyps can occur anywhere in the gastrointestinal tract and can increase in size enough to cause bowel obstruction, most commonly in the small bowel [72].

The *STK-11* gene is located on chromosome 19p13.3 and encodes for serine–threonine protein kinase 11. It is designated as a tumor suppressor gene, participating in membrane bonding and apoptosis [73]. Furthermore, it is a negative regulator of the mTOR pathway [74]. Although PJS has been described since 1949 [75], *STK-11* mutations were identified as its cause in 1998 [76]. Mutations of *STK-11* are detected in approximately 70–80% of patients with PJS, with 15% of them being deletions [77].

Affected individuals are at increased risk for colorectal, breast, small bowel, pancreatic, gastric, and ovarian cancer. Women with PJS present with an increased risk for breast cancer that reaches 50% lifetime. Breast cancer can occur early, but at a lower incidence compared to LFS and CS. In a case series that included 240 patients with PJS, breast cancer incidence has been shown to rise up to 32% by the age of 60, whereas it was only 8% by the age of 40 [78]. Similar to the general population, breast cancer in individuals with PJS is usually ductal in histology. Interestingly, women with PJS also have a 20% risk for ovarian cancer, mainly sex cord tumors [79].

8.4 Moderate-Penetrance Genes

Following the discoveries of *BRCA1* and *BRCA2*, many additional genes have been identified as breast cancer susceptibility genes. A prominent group of these are referred to as moderate-risk susceptibility genes because protein-truncating variants and severely dysfunctional missense substitutions in them appear to confer, on average, two- to fivefold increased risk of breast cancer. This magnitude of increased risk is less dramatic than risks conferred by most pathogenic alleles in the high-risk genes *BRCA1*, *BRCA2*, and *PALB2*, but potentially high enough to influence the medical management of carriers [80]. However, unlike *BRCA1* and *BRCA2*, the risk these genes pose is less certain, although data are accumulating more rapidly because more testing is being done. The most important genes in this group, involved in breast cancer risk, are *CHEK2*, *ATM*, *PALB2*, *NF1*, *BARD1*, *BRIP1*, *MRE11A*, *NBN*, *RAD50*, *RAD51C*, and *RAD51D*.

8.4.1 CHEK2

CHEK2 gene encodes for a serine–threonine kinase, which is activated in response to DNA double-strand breaks. It has also been found to phosphorylate *BRCA1*, facilitating its roles in DNA repair [81]. Certain pathogenic *CHEK2* mutations have been associated with breast cancer. Mutation 110delC has been shown to increase breast cancer risk two- to threefold, while missense mutations have conferred lesser risk [82]. Histologically, 70–80% of *CHEK2*-associated breast cancers are ER-positive [83].

The *CHEK2* 1100delC mutation is particularly frequent in Northern European populations, where it confers a lifetime risk of breast cancer as high as 37% [84]. Homozygotes have a sixfold increased risk of breast cancer [58]. Additionally, some data suggest that *CHEK2* mutation carriers who develop breast cancer have a higher risk of recurrent breast cancers and a poorer disease outcome than noncarriers [85]. Although responsible for less than 1% of familial breast cancer syndromes, *CHEK2* mutations have been identified in approximately 5% of breast cancer patients who are not from BRCA breast cancer families. Furthermore, four other mutations of the *CHEK2* gene have been identified and appear to also confer a moderate breast cancer risk; however only limited data for these four variants are available [86].

8.4.2 ATM

Homozygous *ATM* mutation carriers suffer from ataxia-telangiectasia (AT), a disorder characterized by cerebral ataxia, immunodeficiency, and increased risk of certain malignancies, including breast cancer [87]. Heterozygous carriers of *ATM* mutations have a twofold increased breast cancer risk compared to general population. Women under age 50 with specific *ATM* mutations may have as high as a fivefold increased risk [88]. The risks are particularly difficult to assess because of the high frequency of ATM mutations in the general population.

ATM is a multifunctional gene that plays a pivotal role in double-strand break repair and in cell cycle progression. Genetic testing of *ATM* should be performed in members of families with a known mutation or a history of a clinical diagnosis of ataxia-telangiectasia. ATM is present on most multigene breast cancer susceptibility panels, so will typically be examined in patients with a history of hereditary breast cancer. However, clinical utility of *ATM* genetic testing in heterozygotes is difficult to assess and there are no specific guidelines at this time. Small studies have not demonstrated increased risk of radiation-induced second primary breast cancers, but definitive data on radiation sensitivity are not yet available. Of note, decreased expression of *ATM* protein has been associated with aggressive features in sporadic breast cancer [89].

8.4.3 PALB2

PALB2 has emerged a new breast cancer susceptibility gene that is in transition from moderate to high risk. It was named as a "binding partner and localizer of *BRCA2*," contributing to the DNA repair mechanism homologous recombination and tumor suppression [90]. Classification of *PALB2* as a breast cancer susceptibility gene was based on data showing that about 1% of individuals with hereditary breast cancer negative for *BRCA1/2* harbor a monoallelic mutation in *PALB2* [91]. In a recent study with data from approximately 1500 patients with familial breast cancer, the prevalence of *PALB2* mutations was 0.8%, with the majority occurring in high-risk patients [92]. Although the above studies characterize *PALB2* as a rare, intermediate-risk gene with regard to inherited genetic susceptibility to breast cancer, a recent study that included 154 families with *PALB2* mutations demonstrated a breast cancer risk of approximately 35% [93]. This estimated risk is higher than the one associated with other genes such as *CHEK2* and *ATM* and is classified as high, which may warrant the addition of *PALB2* genetic testing to *BRCA1/2* as a high-penetrance gene for breast cancer, particularly triple-negative disease.

8.4.4 NF1

Neurofibromatosis type 1 is an autosomal dominant tumor predisposition gene with a prevalence as high as one in 2000 births. The pleiomorphic condition is caused by mutations of the *NF1* gene on chromosome 17.3 [94]. *NF1* is a multisystem disease with varying combinations of benign and malignant tumors, developmental dysplasias, and functional deficits, including cognitive impairment. Almost all adult patients with *NF1* have cutaneous neurofibromas, which are benign tumors that do not become malignant. More than one half of patients with *NF1* also have plexiform neurofibromas, which may become malignant [95, 96]. The most common malignancies associated with *NF1* are intracranial gliomas and malignant peripheral nerve sheath tumors (MPNSTs) [97]. In addition to malignancies originating from the nervous system, other cancers associated with *NF1* include breast cancer, gastrointestinal stromal tumor (GIST), and pheochromocytoma [98–100]. Multiple population-based studies have demonstrated a three- to fivefold increase in lifetime breast cancer risk for women with *NF1*, with the highest risks for those <50 years of age. In a study with data from England, the age-specific excess risk of breast cancer comparing the *NF1* cohort with the control cohort was elevated 6.5-fold (95% confidence interval 2.6–13.5) in women aged 30–39, and there was a 4.4 (2.5–7.0) times higher risk among women aged 40–49 [101].

8.4.5 *NBN*, *RAD51C*, *RAD51D*, *BRIP1*, *RAD50*, and MRE11

Genes involved in the Fanconi anemia (FA)-BRCA pathway, critical for DNA repair by homologous recombination, interact in vivo with *BRCA1* and/or *BRCA2* [102]. Some of these genes are mainly associated with ovarian cancer rather than breast cancer, and data are still emerging.

In *NBN*, one protein-truncating variant, c.657del5, is sufficiently common in some Eastern European populations to allow its evaluation in a case–control study. A meta-analysis of ten studies reported strong evidence of an association with breast cancer risk for this variant (summary relative risk, 2.7; 90% CI, 1.9–3.7; $P = 5 \times 10^{-7}$) [103].

For two other DNA repair genes, *MRE11A* and *RAD50*, which encode proteins that form an evolutionarily conserved complex with *NBN*, the data is more conflicting [102, 104, 105]. Currently, there are insufficient data to consider them as breast cancer risk genes [106].

8.5 Multigene Panel Testing

Some considerations must be done when doing multigene panels:

Multipanel can include moderate-penetrance genes for which there are no clear guidelines on risk management for carriers of pathogenic mutations. Until data are clearer, identification of these mutations may not alter the management plan compared to what might be recommended based on family history alone, so their immediate clinical utility could be questioned.

The use of NGS panel testing will lead to a considerable increase in the finding of variants of uncertain significance (VUS), sequence alterations that may or may not affect the function of the gene, or its resultant protein. The frequency of VUS in a large series of clinical specimens examined with NGS breast cancer susceptibility panel reached about 40%, with up to five variants found in individual patients, depending on the series evaluated [11]. An uncertain result—a VUS can ultimately be reclassified as pathogenic or benign—can be very stressful for the patient and family. VUS are clinically troubling for several reasons, including the temptation to assume that a particular VUS is responsible for disease risk in a family, when most will ultimately be considered benign. They are inherited like any other sequence alteration, so it should be shared among family members, but few families are of sufficient size to allow for definitive classification of pathogenicity based on the association with disease status [107]. However, a fraction of VUS will be reclassified to disease causing, highlighting the need for providers to track VUS reclassification and inform patients, which requires time and resources often for many years. Multiple expert groups use functional laboratory assays and computational approaches to classify sequence alterations, which are maintained in publicly supported databases like ClinVar, ClinGen, and the new BRCA Challenge of the Global Alliance for Genomics and Health.

There is a chance of finding a gene that does not match with personal and family history. In some series this finding varies from 0.3 to 0.8% of the tests [11]. Here, the difficulty occurs when mutations are discovered in genes that are predicted to be unrelated to the clinical presentation (e.g., a 40-year-old woman with ductal carcinoma of the breast with no family history of gastric cancer and a mutation in *CDH1*). The appropriateness of counseling this young woman to consider risk-reducing gastrectomy or testing family members for the CDH1 mutation in the setting of concern for gastric cancer risk remains difficult to determine [44].

8.6 Management of Carriers of Mutations in High-Penetrance Genes

8.6.1 BRCA1 and BRCA2

The main goal in management of BRCA mutation carriers is to reduce the risk of developing cancer or at least to promote an early opportune diagnosis and increase the chances of cure.

8.6.1.1 Screening

Breast Cancer

Surveillance of breast cancer in BRCA carriers includes monthly self-examinations, clinical breast examinations twice a year, and yearly magnetic resonance imaging (MRI) of breasts starting at age 25–30 with the addition of annual mammograms thereafter. Earlier screening can be discussed in a family with history of breast cancer prior to age 30. Between ages 25 and 30, MRI is preferred over mammography as false-negative mammogram has been associated with dense breast tissue, and multiple prospective trials have demonstrated far inferior sensitivity of mammogram compared to MRI in BRCA1/2 mutation carriers. In *BRCA1*, the development of "interval cancers" between imaging studies led to the practice of alternating mammograms and MRI's 6 months apart and the recommendation for breast self-exam in *BRCA1* mutation carriers [108]. Between ages 30 and 75, at this time, both breast annual MRI and mammogram are recommended, and after age 75, screening must be individualized [71].

Although earlier studies have not shown an association between radiation exposure and an increased risk of breast cancer in *BRCA* carriers, a recent study did find an increased risk of breast cancer when patients are exposed

to radiation (including mammogram) before age 30. This study further highlights the possible advantage of using MRI alone in this group [109].

Ovarian Cancer

Unfortunately, there is no effective screening for ovarian cancer at this time. The use of transvaginal ultrasound plus CA 125 has not proven to be sufficiently sensitive and specific to substitute for surgery in women at increased genetic risk of ovarian and related cancers.

The NCCN does not consider screening for ovarian cancer to be a reasonable substitute for salpingo-oophorectomy in women with HBOC syndrome [34]. A woman who declines salpingo-oophorectomy can undergo screening with the use of serum measurement of CA 125 and transvaginal ultrasonography every 6–12 months starting at ages 30–35 or 5–10 years before the earliest diagnosis of ovarian cancer in the family, but the patient must be advised about the lack of evidence about this strategy [8]. Ongoing clinical trials are examining bilateral salpingectomies for ovarian cancer risk reduction, with plans for oophorectomies at natural menopause to avoid the very significant side effects of early surgical menopause. Long-term data on efficacy are not yet available.

Other Tumors

Patients with *BRCA1/2* mutations should undergo annually skin examinations as suggested in NCCN and ESMO guidelines because of increased risk of melanoma. There are no official guidelines about pancreatic screening, but the CAPS trials are available for individuals who carry a pathogenic *BRCA1/2* mutation and have a family history of pancreatic cancer in close relatives [8, 71].

8.6.1.2 Risk-Reduction Surgeries

Bilateral Mastectomy

Bilateral mastectomy is largely accepted as an option for women carrying a mutation in either *BRCA1* or *BRCA2*. From 1999 through 2004, the results of four retrospective and prospective observational studies were published. These studies compared breast cancer outcomes in women who underwent prophylactic mastectomy with women of similar risk who did not undergo surgery [110–114]. Four studies showed a **reduction of 90%** or more in the risk of subsequent breast cancer among women who underwent prophylactic mastectomy; updated reports and additional studies have confirmed these initial results.

Although bilateral mastectomies have shown to reduce breast cancer incidence, this procedure is not associated with reduction in breast cancer mortality. This information must be discussed with patients in order to help them decide between surgery and surveillance [113].

Recently, a new technique called nipple-sparing or total skin-sparing mastectomy is becoming more and more common. During this procedure, the surgeon preserves the overlying skin of the nipple areola complex and removes the underlying glandular tissue at risk. Reconstruction can be performed immediately with a variety of techniques. In this procedure, cosmesis is enhanced by preserving the nipple skin, leading to less psychosocial impact, but still loss of sensation and erectile function. More about these surgical options will be addressed in Chap. 10 [115].

Risk-Reducing Salpingo-Oophorectomies

Due to the lack of effective screening for ovarian cancer, *BRCA1/2* mutation carriers are advised to undergo a risk-reducing salpingo-oophorectomy (RRSO) between ages 35 and 40, after women have completed childbearing. This surgery reduces the risk of developing ovarian cancer by as much as 85–96% and breast cancer by approximately 50% [116–119] and reduces both ovarian and breast cancer mortalities [119]. For women with *BRCA2* mutations, who have previously undergone mastectomy, the delay of RRSO until ages 40–45 can be considered since the ovarian cancer onset tends to be late in *BRCA2* carriers [120]. Uptake of RRSO, however, varies widely among individuals and across countries, with lower uptake among European than American women in most data.

Premature surgically induced menopause can lead to an increased risk of cardiovascular disease, osteoporosis, vasomotor symptoms, sleep disturbances, mood swings, and sexual dysfunction and thus adversely affect quality of life. Hormone replacement therapy (HRT) has been shown to at least partially alleviate vasomotor symptoms in *BRCA1/2* mutation carriers and decrease fracture risk in the general population [121–123]. Data have shown that short-term use of HRT in *BRCA1/2* mutation carriers does not negate the breast cancer risk reduction gained by RRSO [124]. The use of HRT may therefore mitigate the adverse effects of surgery on quality of life.

Given the adverse effects of premature menopause and data that ovarian cancer most often originates in the fallopian tube fimbria rather than ovarian surface epithelium, there has been growing interest in salpingectomy with or without delayed oophorectomy as a risk-reducing strategy. It is important to recognize that even with a better profile of impact in quality of life, this approach is not the standard of care of risk-reducing surgery. Fortunately, clinical trials are under way [125, 126]. In addition, oophorectomy in premenopausal women has been shown to reduce the risk of developing breast cancer by 50% in BRCA carriers, depending on the patient age at the time of the procedure, and salpingectomy probably will not have the same effect. These data have recently been questioned.

Chemoprevention

Oral contraceptives, which reduce ovarian cancer risk in the general population, also reduce ovarian cancer risk in *BRCA1/2* mutation carriers, but aspects of the data have been controversial. Observational studies have shown associations between the use of oral contraceptives and a reduced risk of ovarian cancer among *BRCA1* and *BRCA2* carriers, with odds ratios suggesting a 40–50% reduction in risk [127, 128]. However, there are data showing a 2.5-fold increase in breast cancer risk in *BRCA2* carriers in particular, for whom the ovarian cancer lifetime risk is lower (10–20%). The risk reduction with oral contraceptives is generally not considered sufficient to render risk-reducing salpingo-oophorectomies unnecessary, though some women may feel more comfortable delaying surgery if they have used oral contraceptives for many years.

Currently, data on the use of tamoxifen for primary prevention of breast cancer in *BRCA1* and *BRCA2* carriers are very limited. The only prospective data derive from the National Surgical Adjuvant Breast and Bowel Project P1 trial where investigators identified mutation status in 288 women who developed breast cancer, among whom only eight *BRCA1* carriers and 11 *BRCA2* carriers were identified. The hazard ratios for the development of breast cancer among women who received tamoxifen were 1.67 (95% CI, 0.32–10.7) among *BRCA1* carriers and 0.38 (95% CI, 0.06–1.56) among *BRCA2* carriers. Although these results are limited by small sample sizes, they are consistent with an effect in *BRCA2* carriers; approximately 77% of breast cancers in *BRCA2* carriers are ER-positive. Because of small sample sizes, these results are uninformative for *BRCA1* carriers. And the major question of whether or not tamoxifen can provide primary prevention of breast cancer in *BRCA1* carriers, of whom 75–80% of breast cancers are ER-negative, remains. The case–control studies involving *BRCA1* and *BRCA2* carriers reported that tamoxifen protects against contralateral breast cancer with odds ratio of 0.50 (95% CI, 0.30–0.85) to 0.38 (95% CI 0.19–0.74) for *BRCA1* carriers and 0.42 (95% CI, 0.17–1.02) to 0.63 (95% CI 0.20–1.50) for *BRCA2* carriers [129, 130]. Data are more consistent for tamoxifen in the setting of secondary prevention.

There are some preclinical studies suggesting that the use of PARP inhibitors can delay tumor development and extend the life span of *BRCA1*-deficient mice [131], but there are no current trials in humans as these drugs have only limited approval in the therapeutic setting at this time.

8.7 Li–Fraumeni Syndrome

Current practice guidelines established by the NCCN recommend yearly physical examinations including skin and neurologic examinations for all individuals with LFS, with special attention to the possibility of rare malignancies, secondary malignancies, and/or pediatric cancers, depending on the at-risk population. The NCCN also advise some specific considerations as outlined in detail below.

8.7.1 Breast Cancer

Breast cancer is one of the most common cancers occurring among women with germline TP53 mutations. Breast cancer surveillance programs for women with LFS are based on data that established the value of breast MRI in women with *BRCA* mutations.

The complete screening program includes a clinical breast examination once or twice yearly beginning at the age of 20–25. This can be performed earlier depending on the earliest age of onset of breast cancer in the family. Imaging should be used as a screening modality annually starting at the age of 20–25, or earlier, depending on family history. The NCCN recommend that between ages 20 and 29, patients should receive annual MRIs; after age 29, patients should begin mammograms and continue with MRI [71]. European guidelines generally do not include mammography because of concerns about radiation exposure in this population.

There are no data on this specific group regarding risk-reducing surgery, but depending on the prevalence of breast cancer in LFS patients, mastectomy may be considered based on BRCA patient data [71, 132].

8.7.2 Whole-Body MRI

Regarding the large spectrum of tumor in patients with LFS, other strategies have been discussed in order to improve surveillance in this group. A recent study incorporated whole-body MRI into a comprehensive screening protocol that also included clinical examinations and laboratory measures [133]. Participants who elected to enroll in this enhanced screening protocol were compared with those who decided not to undergo screening. The group without screening presented with tumors when they became symptomatic. The striking finding was the difference in outcome between the two groups: individuals in the group who underwent screening had a significant survival advantage with 100% survival at 3 years compared to 21% in the non-surveillance group (95% CI 4–48%) [133]. Separate breast MRI is still required in females because whole-body MRI does not visualize the breasts in sufficient detail.

The use of MRI in this setting has the distinct advantage of avoiding ionizing radiation, and as technology improves, faster whole-body screens have become possible. The existing data, while impressive and hopeful, are neither randomized nor complete; thus whole-body MRI is not yet a standard

of care worldwide. However, multiple research centers are currently working toward the design and implementation of prospective whole-body MRI protocols for LFS families that will contribute to further our understanding regarding the risks and benefits of such screening. Currently, two trials in particular must be considered: the SIGNIFY study in the UK and the LIFESCREEN study in France. Carriers of germline *TP53* mutations should be encouraged to participate in such clinical trials as they are critical for identifying the best care and management strategies for individuals with LFS [132].

8.7.3 Other Tumors

The NCCN also advise consideration of colonoscopy every 2–5 years beginning at age 25 for LFS patients. Annual dermatologic exams are also recommended. Biochemical screening per the Toronto protocol for screening has been less widely adopted [133].

8.8 Cowden Syndrome

8.8.1 Screening

8.8.1.1 Breast Cancer

Women should start MMG and consider MRI, especially in the presence of dense breast tissue, by age 35 or 10 years before the earliest case in the family [71, 134, 135].

8.8.1.2 Other Tumors

As mentioned previously, all these screening procedures are based on expert opinion and have become incorporated into guidelines [71, 134] (Table 8.2). In particular, thyroid surveillance is noninvasive and has been widely adopted.

8.8.2 Risk-Reduction Surgery

8.8.2.1 Mastectomy

Given the high lifetime cancer risks of breast cancer to be 85% [65], prophylactic mastectomy can be discussed on an individual basis, particularly if breast imaging and clinical surveillance are challenging due to extensive benign breast involvement [71, 134].

Table 8.2 Screening procedures for other tumors related to Cowden syndrome

Cancer type	Recommended screening guidelines
Thyroid	Annual ultrasound
Endometrial	Starting at age 30: annual endometrial biopsy or transvaginal ultrasound
Renal cell	Starting at age 40: renal imaging every 2 years
Colon	Starting at age 35: colonoscopy every 2 years
Melanoma	Annual dermatologic examination

8.8.2.2 Hysterectomy

Hysterectomy should be discussed after childbearing, due to the risk of endometrial cancer [71, 135].

8.9 Peutz–Jeghers

8.9.1 Screening

The new guideline from the American College of Gastroenterology suggests that surveillance in affected or at-risk PJS patients should include monitoring for colon, stomach, small bowel, pancreas, breast, ovary, uterus, cervix, and testes cancers, but the guideline does not specify the frequency or the exams that should be performed [136]. Regarding breast cancer risk development, MRI should be considered, but mastectomy is not usually recommended [71].

8.10 Management of Carriers of Mutations in Moderate-Penetrance Genes

There is a lack of evidence regarding management of moderate-penetrance genes. Although these genes are more frequently evaluated by multipanel testing, for the great majority of them, there are no prospective good data about penetrance, management, and clinical utility [71, 137]. The cancer risks associated with mutations in these genes are lower and different than those reported for high-penetrance genes, and the extrapolation of guidelines for the management of individuals with high-penetrance variants of cancer susceptibility genes to the clinical care of patients with moderate-penetrance gene mutations could result in substantial harm [106].

The NCCN guidelines suggest to do breast MRI and/or mammogram. Breast MRI is recommended when lifetime breast cancer risk exceeds 20%, as in patients harboring mutations in *ATM*, CHECK2, and *PALB2*; there are insufficient data for the intervention for *BRIP1*. The guidelines suggest offering RRSO for patients with mutations in *BRIP1*, *RAD51C*, and *RAD51D*; there are insufficient data for *PALB2*. Mastectomy should be offered just to *PALB2*, and the evidence is still insufficient for *ATM* and CHECK2. Table 8.3 summarizes this discussion [71].

Another important consideration is when to start breast cancer screening for these patients. In a recent publication in *Nature Reviews Oncology*, Tung et al. discussed this issue and concluded that screening should begin in this population when the average 5-year lifetime risk exceeds population

Table 8.3 Clinical management guidelines of high-penetrance genes

Gene syndrome	Lifetime risk of breast cancer	Other tumors	Breast cancer screening	Risk-reducing surgery	Other screening
BRCA1/2 HBOC syndrome	50–85%	Ovarian, pancreatic, melanoma, prostate	25–29 MRI 30–75 MMG + MRI	Mastectomy RRSO	–
TP53 Li–Fraumeni syndrome	65–90%	Sarcoma, leukemia, adrenocortical brain tumors, other	20–29 MRI 30–75 MMG + MRI	Mastectomy	Whole-body MRI
CDHI HDGC syndrome	45%	Gastric	30–75 MMG + MRI	Mastectomy Gastrectomy	–
PTEN Cowden syndrome	85%	Endometrial thyroid, colorectal, renal	30–75 MMG + MRI	Mastectomy Hysterectomy	Colonoscopy Thyroid USG TVUS + Endo BX Renal Ultrasound
STK11 Peutz–Jeghers	32%	Colorectal, small bowel, pancreatic, gastric, and ovarian	30–75 MMG + MRI	–	–

Table 8.4 Screening guidelines for moderate-penetrance genes (after Tung et al. [106])

Gene	Mammography (clinical breast examination and/or MRI)	RRSO
ATM	Annual starting at 40 y	Based on family history
CHECK2 truncating	Annual starting at 40 y	Based on family history
NBN	Annual starting at 40 y	Based on family history
PALB2	Annual starting at 30 y	Based on family history
BRIP1	Based on family history	50–55 y
RAD51C	Based on family history	50–55 y
RAD51D	Based on family history	50–55 y

risk at ages 45–50, the age at which mammographic screening is recommended in women in the USA [106]. Women with pathogenic mutations in *PALB2*, *ATM*, *NBN*, and *CHEK2* (other than p.I157T) have a cumulative life risk (CLTR) of breast cancer that exceeds 20% and thus meet existing guidelines for MRI surveillance, at least in the USA. For practical reasons it would be reasonable to initiate MRI surveillance at the same time as mammography [106].

The suggested age to start screening annually with mammogram and/or MRI is 40 years for *ATM*, CHECK2, and *NBN* and 35 years for *PALB2*. The RRSO procedure should be considered only for *BRIP1*, *RAD51C*, and *RAD51D* between ages 50 and 55 [106] (Table 8.4).

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Chemoprevention

9

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9.1 Introduction

In 1976, Sporn defined the term "chemoprevention" as the use of natural, synthetic, or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer [1].

Although the precise mechanisms promoting the development/progression of breast cancer are not completely established, the success of several clinical trials in preventive settings, mainly in selected high-risk populations, suggests that chemoprevention is a rational and an appealing strategy (Fig. 9.1).

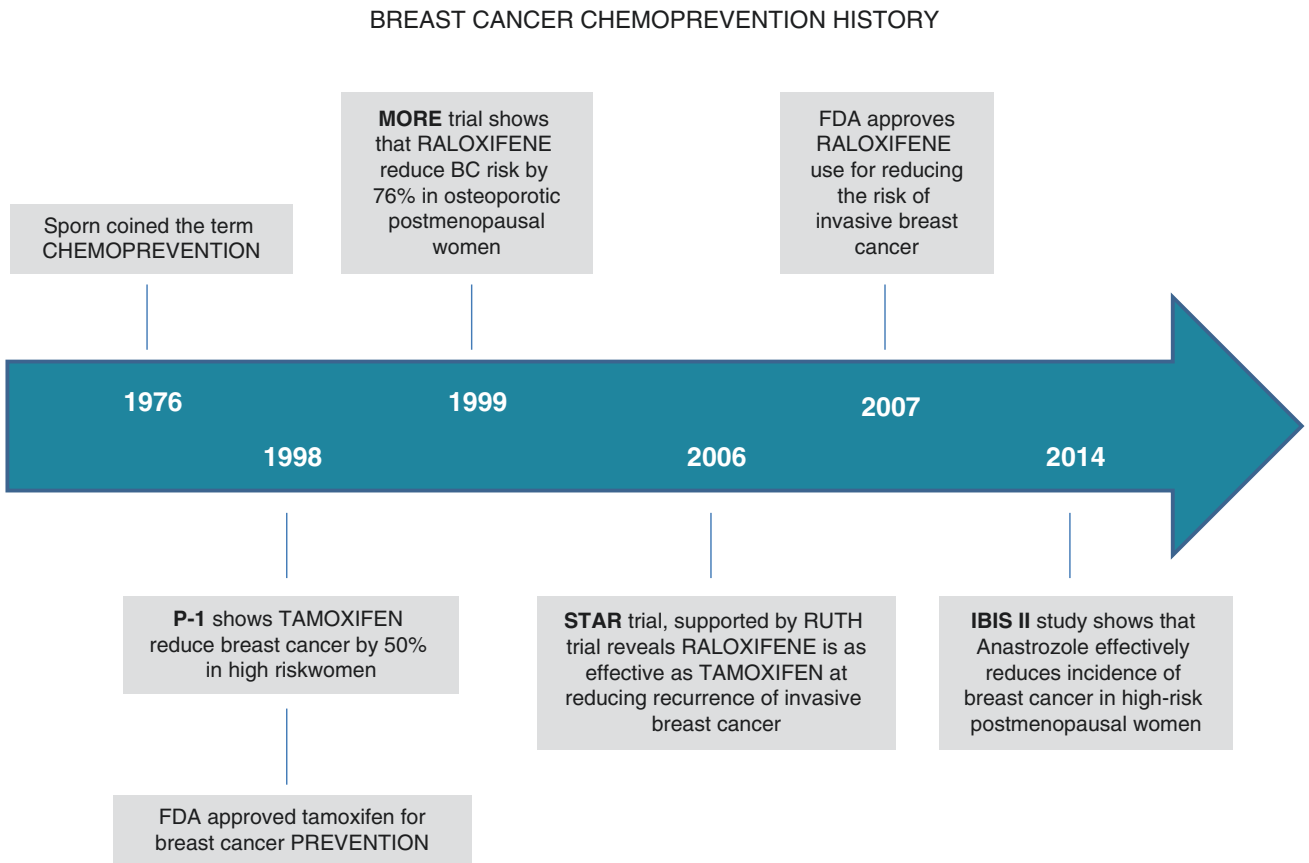

Fig. 9.1 Breast cancer chemoprevention history

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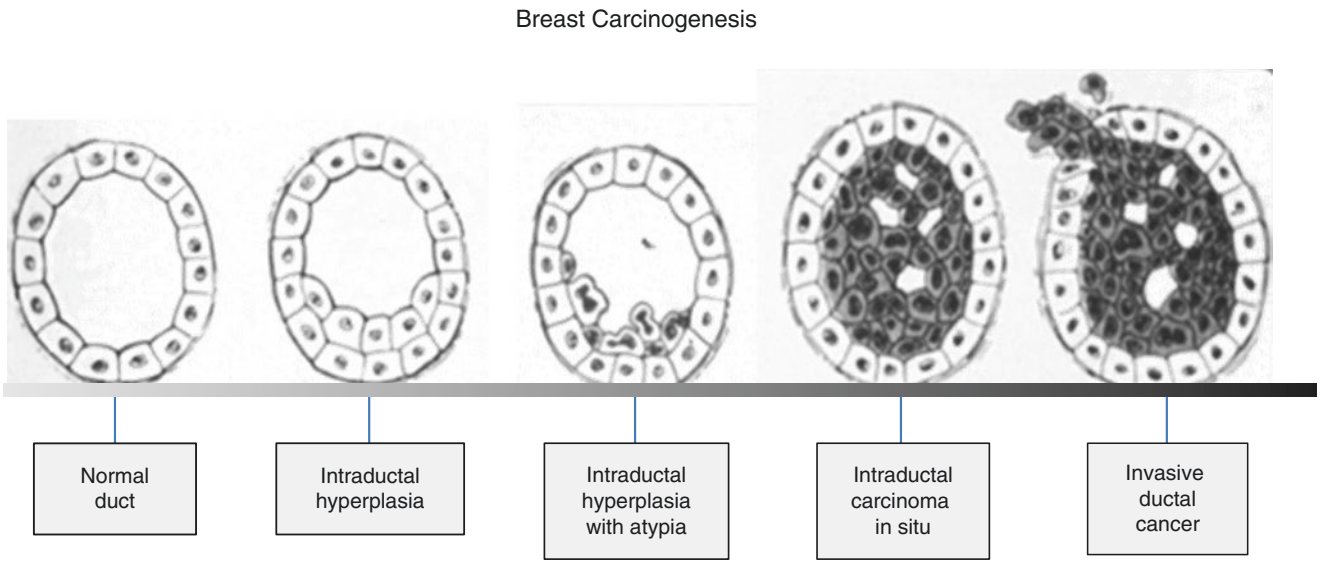

Fig. 9.2 Breast cancer carcinogenesis

Breast carcinogenesis is a multistep, multipath, and multiyear disease of progressive genetic and associated tissue damage. It spans the continuum from simple hyperplasia without atypical cells to intraepithelial neoplasia (IEN) and finally to invasive cancer (Fig. 9.2) [2].

In detail, the carcinogenetic process starts with unspecified accumulations of genetic events leading to a progressive dysplastic cellular appearance with genotypic and phenotypic alterations, deregulated cell growth, and finally cancer. Chemoprevention is just part of these mechanisms and works with the aim to arrest or modify them, thus resulting in a decrease in the incidence of the disease.

In the last few decades, following the therapeutic paradigm for the treatment of cardiovascular diseases that began to include a chemopreventive risk reduction approach [3], preventive therapy for several kinds of cancers, including breast cancer, is currently oriented toward the reduction of modifiable risk factors. The first task, of course, is to identify modifiable factors that would influence the development and progression of the disease in order to tailor prevention strategies on the basis of the individual risk. However, it is now accepted that therapeutic cancer prevention is an effective and essential tool in the fight against cancer, although the use of preventive therapy is sadly still inadequate.

Many subjects at increased risk for breast cancer could benefit from preventive therapy. Defining breast cancer risk incorporates knowledge of individual risk factors known to be associated with increased risk. These risk factors are included in various available risk calculation models, mainly Tyrer-Cuzick and Gail model, to provide a numeric risk that can be used to help quantify the level of individual risk. Other individual risk factors for the selection of candidates for preventive therapy are substantially the presence of premalignant disease (LCIS, ADH, ALH), the presence of mammographic density, and/or the use of HRT, the high-risk penetrant genes (BRCA mutation carriers) or the less penetrant but higher frequency polygenic risk score SNPs [4, 5].

9.2 Breast Cancer Chemoprevention

9.2.1 Prevention of ER-Positive BC

Although the precise mechanism causing breast cancer is not fully established, it is well known that hormones play a significant role in almost 70% of cases [6] and current chemopreventive strategies have targeted hormonally responsive breast cancers. The two major classes of antiestrogenic drugs, selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs), have been recently used for their activity in breast cancer prevention.

The SERM tamoxifen was proven extremely effective on recurrent and new contralateral tumors, hence showing a good toxicity profile in the treatment of hormone receptor-positive breast cancer [7]. Tamoxifen has therefore been an obvious candidate for assessment as a preventive agent.

Four historical large trials [8–11] on tamoxifen effectiveness were undertaken, and long-term follow-up data are available. An overview of these trials has shown a 43% reduction in estrogen receptor (ER)-positive invasive breast cancer, but no effect on ER-negative disease [12]. The data from these studies and, in particular, from NSABP P-1 trial led to the 1998 US Food and Drug Administration (FDA) approval of tamoxifen for reduction of breast cancer incidence in high-risk women.

Furthermore, the direct comparison of tamoxifen with raloxifene (a second-generation SERM) in the STAR trial showed that raloxifene is less effective than tamoxifen (mainly on in situ breast cancer), but with fewer side effects. The initial report from 2006 found raloxifene to be as effective as tamoxifen in preventing invasive breast cancer, but with fewer associated toxicities. In the recent update [13], raloxifene has retained approximately 81% of the effectiveness of tamoxifen in preventing invasive breast cancer and continued to grow closer to tamoxifen in preventing noninvasive breast cancer. Raloxifene has also maintained a better profile with respect to uterine disease, thromboembolic events, and death.

Data from the STAR trial and the other raloxifene/placebo trial (MORE-CORE and RUTH) resulted in the approval of this drug by the US FDA for a reduction in the risk of invasive breast cancer in postmenopausal women with osteoporosis as well as a reduction in the risk of invasive breast cancer in postmenopausal women at high risk of invasive breast cancer. Finally, another recent meta-analysis by Cuzick [14] of nine randomized double-blind trials comparing various SERMs with placebo or another drug on women without breast cancer showed a 38% reduction in breast cancer incidence overall, including ductal carcinoma in situ (Fig. 9.3). The reduction appears larger in the first 5 years of follow-up than in years 5–10. Authors reported a reduction in both year groups, though with a minor effect in the 5–10-year group. No evidence of heterogeneity was found between trials. Moreover, the analysis recorded a significant 34% reduction in vertebral fractures.

While all SERMs increased venous thromboembolic events, only tamoxifen showed a clear increase in endometrial cancers. The large amount of extended follow-up available for this analysis has provided a clear overview of the benefits and harms of these drugs.

The fear of incurring some adverse effects of this drug has hampered its uptake by women at increased risk, and a relative recent route of administration of tamoxifen seems to have solved the question. A simple and economic approach to retain tamoxifen efficacy while reducing the risks was to diminish its dose. The effects of these different doses on proliferation were analyzed using the Ki-67 expression, as the

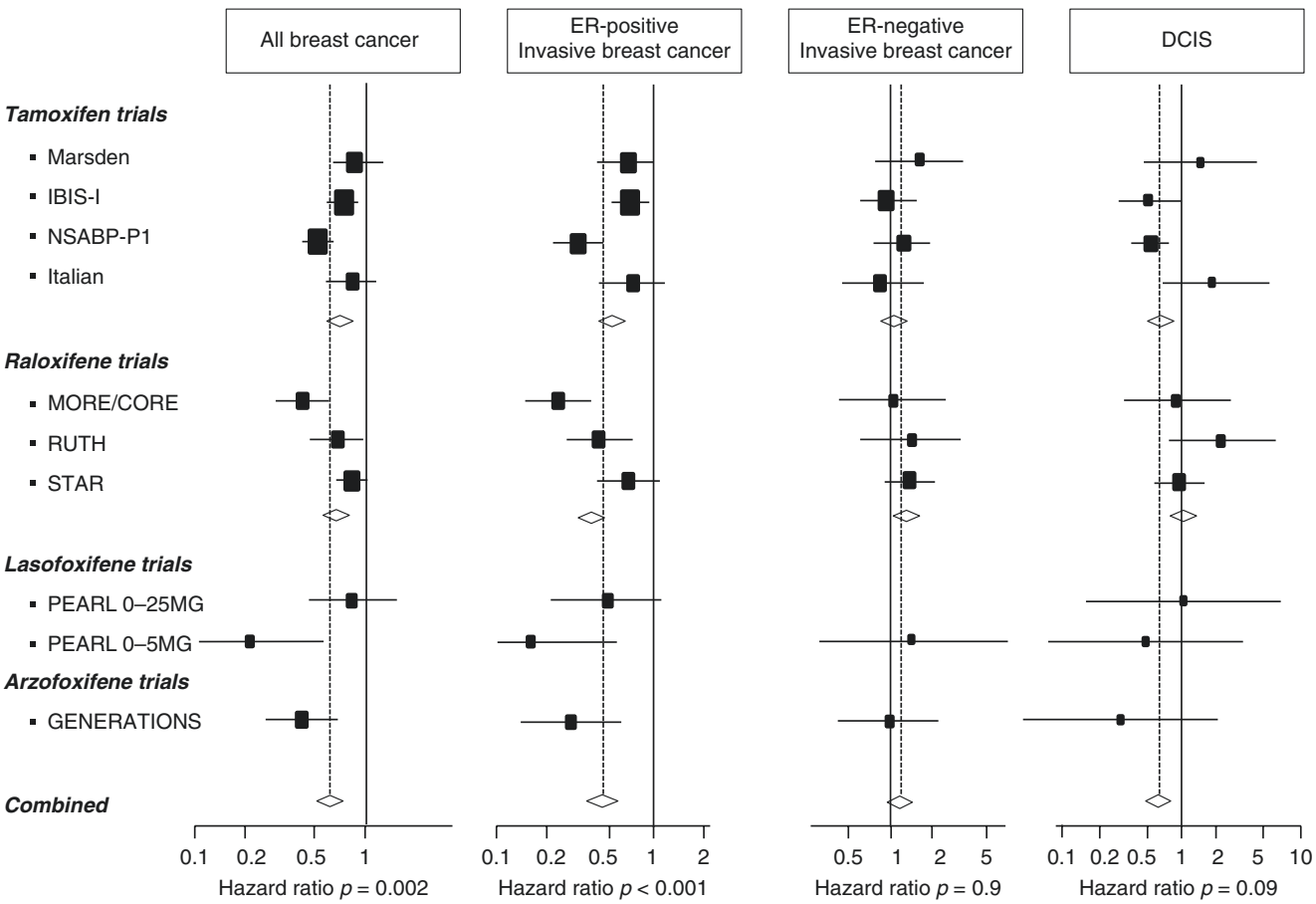

Fig. 9.3 SERMs efficacy in all breast cancer (invasive and in situ) in 10-year follow-up

main surrogate end point marker in several studies [15–18]. The change of the marker expression induced by lower doses of tamoxifen was confirmed to be comparable to that obtained with the standard dose.

Each of the three third-generation AIs used in adjuvant BC trials is effective in suppressing aromatase activity by 97–99% [19], thus achieving near-complete inhibition of aromatase in vivo as well as near-complete suppression of plasma estrogen levels. The significant reduction in contralateral BCs found in adjuvant AI clinical trials [20] has raised interest in these agents for primary prevention, in particular because they may be associated with a less adverse effect profile, specifically in thrombophilic events and endometrial cancer, compared with SERMs. There have been two landmark studies of AIs for BC primary prevention.

The National Cancer Institute of Canada Clinical Trials Group Mammary Prevention 3 (MAP.3) trial was an international prospective, randomized, placebo-controlled, double-blind study, designed to detect a 65% relative reduction in IBC with 25 mg of exemestane compared with placebo [21] In addition, the combined incidence of IBC and DCIS was reduced by 53% in the exemestane group compared with placebo. The IBIS-II [22] was a double-blind, randomized, placebo-controlled study that aimed to assess the efficacy and safety of anastrozole for BC prevention in high-risk postmenopausal women. A total of 3864 postmenopausal women were randomized to either 1 mg of anastrozole daily or placebo. This study showed that anastrozole significantly reduced IBC (mainly high-grade tumors) and DCIS diagnoses.

The MAP.3 and IBIS-II results demonstrate that exemestane and anastrozole were associated with a greater magnitude of BC risk reduction compared to SERMs. However, we should also consider their less adverse effect (especially in thrombophilic and gynecological events) and their simultaneous associated reduced bone mineral density leading to an increased fracture risk, an increase in musculoskeletal side effects, and also, most likely, an increase in cardiovascular events [23, 24]. Relevant issues for both types of chemoprevention, i.e., appropriate duration of therapy, dose optimization, target population, and, ultimately, effects of primary prevention on mortality, still remain unanswered. Finally, because of the absence of head-to-head comparisons and inter-study differences in patient characteristics, it remains unclear whether SERMs or AIs are the preferred agents for BC chemopreventive risk reduction.

9.3 Prevention of ER-Negative BC

Estrogen receptor-negative and triple-negative breast cancers are types of aggressive tumors that account for approximately 30 and 15% of total breast cancers, respectively [25]. Selective estrogen receptor modulators and aromatase inhibitors are unable to treat and prevent these subtypes of mammary tumors, and other approaches are, therefore, needed. Notably, around 90% of breast cancers arising in BRCA-1 mutation carriers are triple negative or estrogen receptor negative [26]. For these reasons, available preventive strategies are urgently needed in BRCA mutation carriers and, in general, in young high-risk population.

It is therefore worth identifying new pathways, biomarkers, and agents that are effective in the treatment and prevention of these subtypes. With the accumulating knowledge in understanding the biology of cancer development, several classes of a new generation of chemopreventive agents modulating the non-endocrine biochemical pathways have been developed, and many of these are still currently under investigation (Table 9.1).

These agents include retinoids, poly(ADP-ribose) polymerase (PARP) inhibitors, EGFR tyrosine kinase inhibitors (for HER2-positive tumors), metformin, cyclooxygenase-2 (COX-2) inhibitors, bisphosphonates, and peroxisome proliferator-activated receptor (PPAR) inhibitors. Due to their lack of proven efficacy or to an unacceptable risk-benefit ratio for healthy subjects, several of these agents are currently on standby. Only the following most apparently promising agents are described.

Table 9.1 Class, specific pathways, and agents actually involved in the treatment and prevention of ER-negative breast cancer

Class	Targets	Drugs or agent
Nuclear receptors	Retinoid acid receptor RXR	Fenretinide (4-HPR) 9 cis-retinoic acid (Targretin)
	VDR	VIT D3 analogues
	$PPAR_Y$	Troglitazone, rosiglitazone, pioglitazone
Membrane receptors and signal transduction	HMG-CoA	Statins
	Tyrosine kinase	Gefitinib (Iressa)
	HER-1, HER-2	Trastuzumab (Herceptin), lapatinib, genfitinib, neratinib
	IGF-R, IGF-1, IGFBP3	Metformin
Anti-inflammatory and antioxidant	COX-2	Celecoxib,rofecoxib, NSAIDs
Angiogenesis	VEGF	Bevacizumab
DNA modulation	BRCA1-BRCA2\	PARP-inhibitors

4-HPR N-(4-hydroxyphenyl) retinamide, *COX* cyclooxygenase, *ER* oestrogen receptor, *HMG-CoA* 3 hydroxy-3 methylglutaryl coenzyme A, *NSAIDs* nonsteroidal anti-inflammatory drugs, *PARP* poly (ADP-ripose) polymerases, *PPAR* peroxisome-activated receptor, *RXr* retinoid X receptor, *VDR* Vitamin D receptor

9.4 Retinoids

Retinoids (either natural or synthetic compounds structurally related to vitamin A) have long been studied for their chemotherapeutic effect and for their chemopreventive potential in breast cancer setting. They are able to regulate cell growth, differentiation, and apoptosis [27] in ER-positive and ER-negative breast cancer cells. The most promising retinoid in chemoprevention setting is fenretinide, N-4-hydroxyphenyl retinamide (4-HPR). The first important study where 4-HPR was administered as a single agent was an Italian multicentric phase III randomized trial, started in 1987. Stage I breast cancer patients were randomly assigned to receive either no treatment or fenretinide given orally at a dose of 200 mg/day for 5 years. The main outcome measure was the occurrence of contralateral breast cancer as first malignant event. Also, a different effect was noticed when the analysis was stratified by menopausal status, with a beneficial trend in premenopausal women on both contralateral and ipsilateral breast cancer (38%) and a reversed trend on contralateral breast cancer in postmenopausal women. Importantly, the protective effect persisted for up to 15 years (i.e., 10 years after retinoid cessation) [28]. Such benefit was associated with a remarkable 50% risk reduction in women aged 40 years or younger. This phase III trial suggested a possible role of fenretinide as a preventive agent acting at different levels of breast carcinogenesis. This protective effect was suggested also in women with a high probability of carrying a BRCA mutation.

9.5 Metformin

Epidemiological studies have strongly suggested that metformin can reduce cancer risk and mortality in diabetic subjects. A recent meta-analysis [29] on 47 independent studies and 65,540 cancer cases in diabetic patients showed that metformin reduced the overall cancer incidence by 31%, while mortality was reduced by 34%. Several preclinical studies have confirmed the effect of metformin in vitro and in vivo and showed a significant reduction of both breast epithelial cell proliferation and protein synthesis [30, 31].

Because of these promising epidemiologic and preclinical data, several phase I and II trials were conducted to investigate its breast cancer preventive effects [32–34]. Most of these were neoadjuvant "window of opportunity" studies among women with operable breast cancer and investigated a variety of biomarker changes after metformin administration. Metformin reduced proliferation (KI67) and increased apoptosis (TUNEL staining) in invasive tumor tissue, in particular in patients with a metabolic unbalanced condition [32, 35]. Phase II and III clinical trials are currently in progress to further elucidate the cancer preventive effect of metformin [36–39]. The most important one is a currently ongoing phase III study (the NCIC-MA.32 trial), testing 5 years of metformin versus placebo among women with early-stage breast cancer [36].

Metformin's antineoplastic mechanisms of action involve several pathways through which the drug acts in direct or indirect mode. In particular, metformin regulates the AMPK/mTOR pathway implicated in the control of protein synthesis and cell proliferation [40]. It has been confirmed that metformin produces a significant repression of cell proliferation and this effect was found to be different in human breast cancer cell lines if related to either positive or negative ERs. A complete cell growth repression was detected in ER-positive cell lines, although only a partial inhibition was detected in ER-negative phenotypes [41]. These data suggest that although ER-negative cells are not as sensitive as ER-positive ones, both of them show a reduction in cell growth under metformin treatment.

9.6 Bisphosphonates

Bisphosphonates are commonly used in patients with breast cancer to reduce skeletal-related events in metastatic disease and to mitigate bone loss associated with cancer therapy in early-stage disease. Antiresorptive agents, including bisphosphonates such as ibandronate, risedronate, and zoledronic acid and the receptor activator of nuclear factor kappa B ligand (RANKL) inhibitor denosumab, have been shown to mitigate aromatase inhibitor-associated bone loss in a series of trials [42]. In addition, adjuvant breast cancer trials evaluating the oral bisphosphonate clodronate suggested a reduction in cancer recurrence and prevention with a direct antitumor effects involving anti-angiogenic, antiproliferative, and proapoptotic mechanisms [43]. Recent adjuvant trials suggest that bisphosphonates may also delay disease recurrence in some populations of estrogen-depleted women in early breast cancer setting supporting a potential anticancer effect. Two large cohort studies reported reductions in breast cancer incidence of around 30% in bisphosphonate users [44, 45]. Both studies reported similar benefits for ER-negative breast cancers.

9.7 EGFR Tyrosine Kinase Inhibitors (TKIs)

Researchers have recently focused their attention on EGFR-HER-1 and EGFR-HER-2 pathways and consequently on TK inhibitors, because the mechanism of resistance to antiestrogen therapy is usually associated with an increased expression of HER-1 and HER-2 receptors.

EGFR is one of a family of four closely related receptors (EGFR or erbB-1, HER-2/neu or erbB-2, HER-3 or erbB-3, and HER-4 or erbB-4) that uses tyrosine kinase activity and contributes to a large number of processes involved in tumor survival and growth, including cell proliferation and inhibition of apoptosis and angiogenesis [46], thus making it an attractive target for cancer prevention. There are two different and concomitant strategies able to inhibit erbB activity. One involves blockade of this activity with monoclonal antibodies (trastuzumab), whereas the second involves the TKIs. TKIs have several advantages over monoclonal antibodies, such as oral bioavailability and potentially less toxicity, and these make them attractive preventive agents [47]. There are two agents tested in this setting, lapatinib and gefitinib.

Lapatinib has been evaluated in several phase II and III trials in various types of breast cancer [48, 49]. Moreover, in prevention setting, it showed a significant delay in the development of ER-negative mammary tumors [50]. This preventive action was seen in premalignant mammary lesions, and this suggests its effectiveness also in the initiation and progression of breast carcinogenesis. Gefitinib showed the ability to suppress ER-negative mammary tumor formation in MMTV-ErbB2 transgenic mice [46], and, despite the results of preclinical and clinical studies, gefitinib recognized ability to inhibit proliferation in early-stage breast cancers and in normal adjacent epithelium remains controversial. This could be the rationale for the use of this compound in prevention trial.

Finally, a mention must be done to neratinib, another irreversible tyrosine kinase inhibitor of HER1, HER2, and HER4, which has recently shown [51] clinical activity in patients with HER2-positive metastatic breast cancer. Neratinib for 12 months significantly improved 2-year invasive disease-free survival when given after chemotherapy and trastuzumab-based adjuvant therapy to women with HER2-positive breast cancer. Disease-free survival including ductal carcinoma in situ was also significantly improved with neratinib compared with placebo after 2 years, and this action about early phases of cancinogenesis should be promising in the preventive settings too.

9.8 Limited Uptake of BC Chemoprevention

Despite the availability of several efficacious agents, the utilization of preventive therapy has been poor due to various barriers, such as the lack of physician and patient awareness, fear of side effects, and licensing and indemnity issues. For preventive therapy, we cannot identify those individuals whose cancer was prevented or risk was substantially reduced because of the lack of measurable biomarkers of efficacy, which currently exist for other diseases, including cardiovascular diseases, prevention of diabetes complications, or osteoporotic bone fractures.

Therefore, from those persons' point of view, they either have taken unnecessary medication or, worse, they have unnecessarily suffered the adverse effects of such therapy. Preventive therapy for cancer is often discounted as overtreatment and used as an example of overmedicalization. Understanding and overcoming such perception differences, along with other barriers, are essential if we are to realize the full potential of this approach for cancer control. New strategies are needed in order to improve this condition, and they include improving physician awareness and countering prejudices by highlighting the important differences between preventive therapy and cancer treatment. Researchers in the last few decades have discussed about the important barriers to therapeutic cancer prevention and the strategies to overcome these barriers and future research needs (Table 9.2).

Several reasons seem to be the causes that complicate the spread of the use of preventive therapies, although the most important often seems to be the fear of side effects. Moreover, future research to improve therapeutic cancer prevention needs to include improvements in the prediction of benefits and harms and improvements in the safety profile for new or existing agents by experimentation with dose.

Table 9.2 Barriers to preventive therapy and strategies to overcome these barriers

Barriers	Strategies to overcome barriers
Underestimation of benefits and/or overestimation of harms	• Acknowledging different needs of risk prediction for different diseases and agents • Refining risk prediction and risk communication • Development of biomarkers than can be frequently monitored by non-invasive means
Adverse effects of agents	Exploring strategies to reduce adverse effects, e.g., dosing modifications
Individual lack of knowledge	Improving physician-patient communication and information sharing; educational interventions
Individual's fear of side effect	Exploring re-purposing of commonly used agents with well-documented safety profile
Physicians' lack of knowledge/prejudices	Increasing physician awareness and countering prejudices
Licensing and off-label use issues	Policy engagement
Lack of well-proven agents for several cancers	Increased focus on preventive research, particularly in academia

9.9 Natural Compounds

Lifestyle changes do offer an important strategy for cancer prevention [52]. They generally include diet and nutrition modifications as well as a regular and suitable physical activity. Moreover, recent attention has been given to the use of natural products in a preventive setting, especially in trying to counteract the concern of drugs' side effects, in addition to making a possible preventive approach intriguing [53]. Some of the most promising compounds include catechins (e.g., epigallocatechin gallate (EGCG), green tea extract), curcumin, carotenoids, omega-3 fatty acids, resveratrol, soy isoflavones, and vitamin D. Unfortunately, none of these dietary agents has been shown to consistently prevent breast cancer. So, in spite of the fact that natural products are a promising alternative strategy for cancer prevention, their potential efficacy in the prevention of ER-negative and, particularly, triple-negative breast cancer will be determined in the near future. In particular, it might be useful to identify those natural products that cannot act directly on carcinogenic mechanisms but on the main risk factors. Since the properties of some carcinogenic pathways, such as inflammation, cholesterol, metabolic syndrome, and hyperinsulinemia, are already known, natural products could successfully be used in the regulation and/or control of these pathways and could also indirectly act on the risk of developing the disease.

One simple strategy is to combine nutraceuticals which are in common use as food ingredients to make a single cancer polypill. This was done with success for antihypertensive agents in the polypill for stroke prevention in the general population [54].

Conclusions

The success of several recent clinical trials in the preventive setting in selected high-risk populations suggests that chemoprevention is an effective strategy. New pathways, biomarkers, and agents are actively searched in this subgroup of cancers and have been recently put under investigation in order to improve the effectiveness and reduce the toxicity. These strategies accompanied by a serious lifestyle and nutrition changes could be a decisive step to breast cancer prevention and treatment.

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Surgical Prevention

10

Paolo Veronesi and Nickolas Peradze

The surgical strategies available today for breast cancer prevention are prophylactic bilateral or unilateral mastectomy and prophylactic bilateral salpingo-oophorectomy.

Mastectomy is intended to remove breast tissue and therefore to remove the very substance of cancer growth, while oophorectomy eliminates the major part of the body's source of estrogen, with the additional benefit of decreasing ovarian cancer risk in *BRCA1* and *BRCA2* mutation carriers.

Overall mastectomy rates have increased over the past decades [1–3]. In part, this is due to the introduction of new imaging modalities capable of detecting additional breast lesions, which in turn require more extensive surgery. It is also due to the growing patient choice for preventive surgery as a reflection of the increased awareness of genetic breast cancer, increased genetic testing, and increased knowledge of improvements in mastectomy and reconstruction techniques, together with anxiety and overestimation of the risk of developing breast cancer.

Several studies have demonstrated that the risk as perceived by patients of developing breast cancer is much higher than the objective risk, given the fact that those patients who are the most well informed and involved in decision-making are more likely to choose mastectomy [4, 5].

Mastectomy, especially bilateral, is an extended and elective surgery and may have unfavorable effects in terms of complications and associated costs as well as in terms of body image and sexual function. Therefore a better understanding of its indications, use, and outcomes is crucial to improve cancer care [6–9].

In general, prophylactic mastectomy may occur in two populations of patients: those already affected by unilateral breast cancer who undergo contralateral mastectomy for the prevention of cancer development in the contralateral breast and those without breast cancer, but at high risk, and therefore undergo bilateral risk-reducing mastectomy.

In 2007 Giuliano et al. stated the potential indications for prophylactic mastectomies. In this statement, the indications for prophylactic mastectomies include BRCA mutations or other susceptibility genes, strong family history without genetic mutation, histologic risk factors, difficult surveillance, and reconstructive issues (symmetry/balance) [10].

10.1 Contralateral Prophylactic Mastectomy

Various studies have evaluated trends, implications, and outcomes of contralateral prophylactic mastectomy (CPM).

The Surveillance, Epidemiology, and End Results Program (SEER) from 1998 to 2003 in the USA confirmed increased rates of CPM by 150% [11]. Similar trends were observed in other studies [12–15].

The categories of patient who may benefit from CPM may be varying.

Despite limited evidence in survival improvement after CPM published in 2010 in a large Cochrane analysis [16], several studies which were subsequently published showed minimal benefit in overall survival [17–19].

Yao et al. examined the effect of CPM on survival on 219,983 mastectomy patients using the National Cancer Data Base (the largest study so far to examine survival with CPM). Adjusted hazard ratio (HR) was 0.88 (95% CI 0.83–0.93; $p < 0.001$), and an absolute 5-year benefit of 2% was observed. Differential effect of CPM by stage and age was observed with HR = 0.88 (95% CI 0.82–0.94; $p < 0.001$) in women younger than 70 with stage I/II, and HR = 0.95 (95% CI 0.88–1.04; $p = 0.28$) in women with stage III or older than age 69 with absolute 5-year benefit of 1.3%. This improvement in survival could be attributed to the category of high-risk patients (family history and/or BRCA mutation carriers), who have higher risks of contralateral cancers and therefore may benefit from a CPM. Finally, the authors were unable to establish a cause-and-effect relationship between CPM

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and survival, due to the lack of data regarding family history, BRCA carrier status, hormonal receptor, and HER2 status [20].

In large meta-analysis performed by Fayanju et al., the authors concluded that patients without known high risk (familial/genetic) should not be advised to undergo CPM [21].

In the recent study conducted by Basu et al. [22], the authors developed a series of guidelines to aid clinicians dealing with requests for, and management of, CPM. These included several steps for the process of preoperative assessment and counseling before CPM can be given and may be summarized as follows:

- Taking a history
- Calculating the risk of contralateral breast cancer
- Cooling-off period whenever possible
- Multidisciplinary team (MDT) discussion
- Patient consent

Therefore breast cancer patients should be provided with precise and accurate information on the risk of contralateral breast cancer and on the risks and benefits of CPM.

10.2 Bilateral Prophylactic Mastectomy

As outlined earlier, indications for bilateral prophylactic mastectomies include BRCA1/BRCA2 mutation or other susceptibility genes, strong family history without genetic mutations, and histologic risk factors.

Large prospective analyses report the average cumulative risks for breast cancer by the age of 70 years for BRCA1 carriers of 60% (95% confidence interval [CI] = 44–75%) and 83% (95% CI = 69–94%) for contralateral breast cancer. For BRCA2 carriers, the corresponding risks are 55% (95% CI = 41–70%) and 62% (95% CI = 44–79.5%) for contralateral breast cancer [23, 24].

Retrospective analyses of the results of the study conducted by Hartmann et al. demonstrated risk reduction of about 90% for BRCA1 and BRCA2 mutation carriers after bilateral prophylactic mastectomy (BPM) [25, 26].

In the study conducted by Heemskerk-Gerritsen et al., authors evaluated breast cancer incidence, all-cause mortality, and breast cancer specific mortality in healthy BRCA1 and BRCA2 mutation carriers undergoing bilateral prophylactic mastectomy. These were compared with a surveillance group. No incidence of breast cancer cases was observed during 1379 person-years of observation after BPM. 10-year overall survival was 99% for the BRRM and 96% for the surveillance groups, respectively. The authors concluded that BPM substantially reduces breast cancer occurrence in healthy BRCA1/BRCA2 mutation carriers. However, longer follow-up and larger sample size are needed to confirm statistical significance [27].

Interesting results were presented by the study of Skytte et al., which included 307 women with a pathogenic BRCA1 or BRCA2 mutation, of whom 96 underwent bilateral risk-reducing mastectomy. None of the study participants had a previous history of breast or ovarian cancer or had undergone risk-reducing bilateral salpingo-oophorectomy prior to the time of BRCA testing. The annual incidence of post-mastectomy breast cancer was 0.8% compared with 1.7% in the non-operated group [28].

A more recent meta-analysis performed by De Felice et al. reported risk reduction of developing breast cancer in BRCA1 and BRCA2 mutation carriers after risk-reduction mastectomy by 93% [29].

The risk of breast cancer after mastectomy could be presumably explained by a tumor developing in left-over breast tissue. However this hypothesis is debatable. In fact in the study of Skytte et al., women who developed breast cancer after mastectomy had undergone a simple mastectomy including removal of the nipple–areola complex.

Today the most popular prophylactic mastectomy technique is the so-called conservative mastectomy or nipple-sparing mastectomy/skin-sparing mastectomy or subcutaneous mastectomy with immediate reconstruction. This surgical technique has been shown to be feasible and safe, with outstanding cosmetic results, and allows preservation of the woman's body image [30–33].

This was also supported by a recent review by van Verschuer et al. in which the authors stated that the incidence of primary breast cancers after prophylactic mastectomy is very low after total mastectomy as well as after conservative mastectomy [34]. It is suggested to surgeons that they minimize risk by paying particular attention to ensure that all glandular tissue is dissected, especially in the axillary tail, chest wall, and nipple–areola complex.

Recently Toesca et al. reported the first experience of robotic prophylactic nipple-sparing mastectomy. This seemed to improve outcomes of mastectomy from a cosmetic and patient satisfaction point of view [35].

NCCN guidelines support the use of risk-reducing mastectomies for selected patients at high risk who desire this intervention. Nevertheless histologic factors such as ADH, LCIS are associated with an increased risk of breast cancer, surgical risk reduction is not recommended in most of these patients [36].

There is a lack of data concerning the utility of sentinel lymph node biopsy (SLNB) during risk-reducing surgery. It is recommended that MRI assessment be performed prior to risk-reducing surgery as some patients may be at risk of occult breast cancer, having strong family history or mammographic density, and the use of SLNB should be decided on a case-by-case basis [37].

10.3 Bilateral Prophylactic Salpingo-Oophorectomy

Several studies have reported a breast cancer risk reduction of approximately 50% after risk-reducing salpingo-oophorectomy in BRCA1/BRCA2 mutation carriers [26, 38–43].

Heemskerk-Gerritsen et al. revised the association between risk-reducing salpingo-oophorectomy (RRSO) and breast cancer risk in BRCA1/BRCA2 mutation carriers, proposing a different analytical approach in order to mini-

mize potential biases as much as possible compared with previous studies. Applying the requirement of no history of cancer at the date of DNA diagnosis and the inclusion of person-time preceding risk-reducing salpingo-oophorectomy, the authors found no evidence for first breast cancer risk. Nonetheless, cumulative breast cancer risk curves suggest a slightly protective effect of risk-reducing salpingo-oophorectomy on breast cancer risk when performed at premenopausal age [44].

BRCA1 tumors have been more frequently found to be steroid hormone receptor-negative, rather than BRCA2 tumors [45, 46]. Therefore, the BC risk-reducing effect of RRSO may be expected more in BRCA2 mutation carriers.

In the most recent review conducted, Hartmann et al. summarized and presented current guidelines and statements on indications for preventive surgical procedures [47] (See table provided by Hartmann et al., NEJM).

In conclusion, risk-reducing surgery provides considerable benefits in terms of cancer prevention. Although, being an extended surgery, risk-reducing procedures may be associated with complications and adverse physical and psychosexual effects. Patient education plays an important role in avoiding the overestimation of breast cancer risk. Efforts should be made to provide information regarding sexuality, body image, reconstruction techniques, fertility, and the likelihood of familial predisposition.

The decision to undergo preventive surgical procedures is complex and patients require careful assessment in a multidisciplinary setting comprising clinicians, psychologists, and geneticists.

Box 1. Overview of Key Positions Regarding Risk-Reducing Surgery in Women with Hereditary Breast and Ovarian Cancer Syndrome.

The following position statements pertain to women without prior breast or ovarian cancer. These statements acknowledge that bilateral risk-reducing mastectomy and salpingo-oophorectomy have potential adverse effects, and multidisciplinary consultations before surgery are recommended to ensure informed decision making by the patient.

Bilateral Mastectomy

NCCN (National Comprehensive Cancer Network; www.nccn.org): "Risk-reducing mastectomy . . . provides a high degree of protection against breast cancer in women with a *BRCA1/2* mutation." Discuss risk-reducing mastectomy on a case-by-case basis, with a review of the potential adverse effects of the procedure. Risk-reducing mastectomy is also an option for patients with the Li–Fraumeni syndrome and the Cowden syndrome. Consensus recommendations are not provided for carriers of mutations in other genes.[34]

USPSTF (U.S. Preventive Services Task Force): "Among high-risk women and mutation carriers, risk-reducing mastectomy [as compared with no surgery] decreased breast cancer by 85 to 100% and breast-cancer mortality by 81 to 100%."[35]

Society of Surgical Oncology: Indications for bilateral prophylactic mastectomy include mutations in *BRCA1*, *BRCA2*, or other strongly predisposing breast-cancer susceptibility genes or, in the absence of data on mutations, a hereditary breast-cancer syndrome.[36]

NICE (National Institute for Health and Care Excellence; United Kingdom): "Bilateral risk-reducing mastectomy is appropriate only for a small proportion of women who are from high-risk families and should be managed by a multidisciplinary team . . . . Bilateral mastectomy should be raised as a risk-reducing strategy option with all women at high risk."[37]

Additional international guidelines have been summarized by Easton et al.[10]

Comments on the procedure: No mastectomy can remove all breast tissue, which is widely distributed on the chest wall. Several mastectomy approaches have been used for prophylaxis. A total (simple) mastectomy removes more than 95% of breast tissue, including the overlying skin and nipple–areolar complex. In a classic subcutaneous mastectomy, the skin and nipple–areolar complex are preserved, and varying amounts of glandular tissue may be left below the areola. The use of this procedure for prophylaxis has been criticized because of the possible retention of excess at-risk tissue in the skin flaps and below the areola. Most surgical oncologists recommend a skin-sparing mastectomy for prophylaxis; this preserves the natural skin of the breast. A recent technique called "nipple-sparing" or "total skin-sparing" mastectomy also preserves the overlying skin of the nipple–areola complex. The underlying glandular tissue at risk is removed, and immediate reconstruction is performed. Cosmesis is enhanced by preserving the nipple skin.[38,39] More than 90% of women who undergo bilateral risk-reducing mastectomy elect immediate breast reconstruction, usually with implants. Complications may be immediate or delayed. In a prospective cohort of 112 consecutive women who underwent risk-reducing mastectomy followed by immediate breast reconstruction and were followed for 2.8 years, 10% had bleeding, 9% infection, and 14% capsular contracture. A total of 33% of women required reoperation.[40]

Bilateral Salpingo-Oophorectomy

NCCN: "Recommend risk-reducing salpingo-oophorectomy (ideally in consultation with a gynecologic oncologist) typically between 35 and 40 years, and upon completion of child bearing."[34]

USPSTF: "Risk-reducing salpingo-oophorectomy decreased breast cancer incidence by 37 to 100%, ovarian cancer by 69 to 100%, and all-cause mortality by 55 to 100%."[35]

Society of Gynecologic Oncology: "The most proven method for the prevention of ovarian cancer in women who carry a deleterious *BRCA1* or *BRCA2* mutation is risk-reducing salpingo-oophorectomy. Prospective studies have reported a 70% to 85% reduction in ovarian cancer . . . risk-reducing salpingo-oophorectomy between the ages of 35 and 40 years is recommended for risk reduction in women at increased genetic risk of ovarian cancer. The age [at which risk-reducing salpingo-oophorectomy is performed] may also be individualized according to the earliest age of onset in the family and personal choices."[41]

Comments on the procedure: The procedure, usually performed laparoscopically, should include visual assessment of the abdomen and pelvis, a pelvic washing, and total bilateral salpingo-oophorectomy, including ligation of the ovarian artery and vein approximately 2 cm proximal to the ovary and tube to ensure removal of all tissue. Because of the possibility of occult cancer, including serous tubal in situ carcinoma, meticulous processing of the surgical specimen is necessary according to the SEE-FIM protocol (protocol for sectioning and extensively examining the fimbriated end).[41-43]

Salpingectomy Alone

NCCN: "Salpingectomy [alone] is not the standard of care and is discouraged outside a clinical trial. The concern for risk-reducing salpingectomy alone is that women are still at risk for developing ovarian cancer."[34]

Society of Gynecologic Oncology: "Salpingectomy can be considered at the completion of childbearing in women at increased genetic risk of ovarian cancer who do not agree to salpingo-oophorectomy. However, this is not a substitute for oophorectomy, which should still be performed as soon as the woman is willing to accept menopause, preferably by the age of 40 years."[41]

New England Journal of Medicine, 2016, by Hartmann et al.

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Part III

Pathology: Standard and Molecular Diagnostics

Premalignant and Pre-invasive Lesions of the Breast

11

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11.1 Introduction

Premalignant and pre-invasive lesions of the breast belong to a complex and heterogeneous group of lesions and represent a matter of remarkable interest from both clinical and biological standpoints. These frequent noninvasive alterations are related with an increased probability of breast cancer development. What is more, they show extremely variable risks of progression toward invasive forms of disease. Indeed, while there are many histologically defined premalignant lesions in the breast, only a few of them constitute true neoplastic precursors that will progress to invasive cancer. Disappointingly, it is currently not conceivable to identify a priori, with absolute certainty, which of these precursors will progress and which not. Therefore, classifying risk indicators, precursors, and non-obligate precursors of invasive breast cancer, and ultimately defining robust protocols for their clinical management, is a hot topic in the multidisciplinary approach to breast cancer patients, that involves pathologists, radiologists, surgeons, and oncologists.

The introduction of mammography-based breast cancer screening programs has increased spectacularly the detection of noninvasive lesions [1]. Nowadays, in situ carcinomas account for up to 21% of breast cancer diagnoses [1], while atypical lesions are "incidental" findings in nearly 10% of breast biopsies with non-carcinomatous alterations [2]. Therefore, the correct and early diagnosis of premalignant and pre-invasive lesions of the breast has become one of the most crucial tasks for radiologists and pathologists, given its decisive implications in terms of tailored management schemes implementation. However, the histological identification and classification of this vast collection of entities remains not trivial at all, since the rules for their diagnosis has changed over the time, and even changes over the space with substantial interobserver and inter-institutions variability. Nowadays, breast care providers are living in an era of extraordinary changes in clinical approaches to nonmalignant lesions, and the integration of multiples disciplines as well as cutting-edge diagnostic methods has not yet fully achieved. As a result, guidelines for the screening, treatment, and follow-up of these patients are in constant evolution and reshape, based on the biologic insights that high-throughput technologies are currently providing. At present, to master noninvasive and pre-invasive changes of the breast at both morphologic and molecular levels is key to allow the most appropriate clinical workup for these women.

Over the past few years, many models have been put forward to unravel the complexity of breast cancer tumor progression. To date, it is widely recognized that a strict morphology-based approach is no longer able to capture the innumerable ramifications of this complicated issue. Lately, based on the activation of estrogen receptor (ER) and ER-regulated genes, a multistep model of breast cancer evolution, encompassing most of the precursor and non-obligate precursor lesions, has been proposed [3]. High-throughput sequencing studies are further corroborating this hypothesis [4–6]. However, given the intrinsic limitations of the published studies, including the relatively small sample size and the extremely challenging set up of functional models of breast tumor progression, the biological determiners of the progression from pre-invasive to invasive disease have yet to be fully elucidated. Indeed, we are still not able to predict which lesions will progress to invasive disease. The implementation of novel biomarkers to diagnose and predict the outcome related to noninvasive breast lesions at an individualized level represents the prerequisite for the realization of the potentials of precision medicine.

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11.2 Classification of Noninvasive Lesions of the Breast

11.2.1 Multistep History of Breast Cancer Evolution Model

The initial morphology-based classification systems of breast alterations allowed for several speculations on the cell from which breast malignancies originate. In the past, pioneer scientists postulated that breast cancers would arise from distinct sites within the mammary gland. It was a rather dichotomic but extremely charming conception that soon became viral among breast care providers. Basically, some tumors were thought to originate from the ducts, while others would arise from the milk-producing lobules and therefore named invasive ductal carcinomas and invasive lobular carcinomas, respectively. Not surprisingly, lesions that were morphologically confined inside of their "original site," without evidence of spread to surrounding tissues, were named ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). At present, this "vintage" nomenclature remains widely adopted for indicating adenocarcinoma-like (ductal) and discohesive-cell tumors (lobular). Over 30 years ago, this model has been questioned by the outstanding works of Wellings and Jensen [7, 8] that provided the first scientific evidence that most of breast cancers and a proportion of precursors lesions (both of ductal and lobular types) would arise within the same microanatomical site, namely, the terminal duct lobular unit (TDLU). It is interesting to note that only in 2012, the World Health Organization (WHO) in its classification of breast tumors changed the terminology of "invasive ductal carcinoma, not otherwise specified (NOS)" into "invasive carcinoma of no special type (NST)," avoiding outdated assumptions on the ductal origin of the cancer cells [9]. Drs. Wellings and Jensen proposed a linear multistep model of the breast cancer evolution based on epidemiological data and on the histological continuum of the lesions [7, 8, 10]. The key steps of this model included progression from hyperplasia, to atypical hyperplasia (ductal or lobular), carcinoma in situ (DCIS or LCIS), and ultimately invasive ductal or lobular carcinoma.

During the last decades, the accumulation of new molecular data have further complicated this model of tumor evolution. Indeed, comparative genomic hybridization (CGH) and microarray-based expression profiling studies demonstrated that estrogen receptor (ER)-positive, HER2-positive, and triple-negative (i.e. ER-negative, progesterone receptor (PR)-negative and HER2-negative) breast cancers constitute biologically and clinically distinct diseases [11–13]. In recent years, the advent of next-generation sequencing methods showed that these entities are underpinned by distinct repertoires of genetic aberrations [14]. Seminal analyses of bona fide precursors of breast cancer suggest that these lesions are at least as heterogeneous as their invasive counterparts [3]. It is currently accepted the notion that low-grade and high-grade tumors and their respective precursor lesions harbor completely different genomic and transcriptomic features and evolve through distinct pathways [4, 5, 15–17]. Moreover, molecular analyses identified two distinct pathways of breast cancer evolution based on the activation of ER. The concept of low-grade ER-positive breast neoplasia family encompasses pre-invasive and invasive lesions, including flat epithelial atypia (FEA), ER-positive DCIS, lobular neoplasia, and ER-positive low-grade invasive breast cancers that have been demonstrated to coexist at a frequency greater than expected by chance and to share specific genetic aberrations (e.g., deletions of 16q, gains of 1q and 16p) [3, 18, 19]. Deletion of 16q is an uncommon finding in HER2 positive or triple negative tumors; however, this peculiar genetic signature has been described in a subset of high-grade ER-positive lesions, suggesting a progression from low- to high-grade ER-positive cancers [20]. Recently, microglandular adenosis (MGA) emerged as bona fide non-obligate precursors of both low- and high-grade triple-negative breast cancers, which are characterized by recurrent *TP53* mutations and a complex pattern of somatic mutations and copy-number alterations [21–24]. At present, the combination of nuclear grade and ER status seems to delineate the two major pathways of breast cancer evolution. However, quoting Kornelia Polyak quoting Johann Wolfgang Von Goethe: "*progress has not followed a straight ascending line […]*" [25].

11.2.2 Terminology and Classification Systems

The constantly evolving model of breast cancer evolution leads to continuously reshape the taxonomy of noninvasive breast lesions (Table 11.1). At least three systems are used to classify these entities based on prognostic, predictive or pathologic purposes, and considerations.

In the "prognostic system," the multitude of proliferative breast changes can be categorized as risk indicators (i.e., lesions that are associated with increased risk of breast cancer development in the ipsilateral or contralateral breast) and/or precursors (i.e., lesion that can progress to invasive cancer). Atypical hyperplastic lesions and carcinoma in situ have been shown to confer a relative risk of breast cancer development of 4% [26–30] and 8–10% [3, 31], respectively. Although the low relative risk of atypical lesions have been recognized since the seminal study of Dupont and Page [26, 27], new data on cumulative risk of breast cancer among women with atypical hyperplasia (cumulative risk, 30% at

Table 11.1 Taxonomies of premalignant and pre-invasive lesions

Traditional terminology	WHO 2012 classification	Tavassoli classification	European guidelines for quality assurance in breast cancer screening and diagnosis	Clinical implication (prognosis)
Columnar cell change (CCC) Columnar cell hyperplasia (CCH)	Columnar cell lesion (CCL)		B2	Risk indicator and non-obligate precursor
Columnar cell change with atypia (A-CCC) Columnar cell hyperplasia with atypia (A-CCH)	Flat epithelial atypia (FEA)	Ductal intraepithelial neoplasia, grade 1A (DIN 1A)	B3	Risk indicator and non-obligate precursor
Atypical ductal hyperplasia (ADH)	Atypical ductal hyperplasia (ADH)	Ductal intraepithelial neoplasia, grade 1B (DIN 1B)	B3	Risk indicator and non-obligate precursor
Ductal carcinoma in situ, low grade (DCIS grade 1) Ductal carcinoma in situ, intermediate grade (DCIS grade 2) Ductal carcinoma in situ, high grade (DCIS grade 3)	Ductal carcinoma in situ (DCIS), low grade Ductal carcinoma in situ (DCIS), intermediate grade Ductal carcinoma in situ (DCIS), high grade	Ductal intraepithelial neoplasia, grade 1C (DIN 1C) Ductal intraepithelial neoplasia, grade 2 (DIN 2) Ductal intraepithelial neoplasia, grade 3 (DIN 3)	B5	Risk indicator and non-obligate precursor
Atypical lobular hyperplasia (ALH)	Atypical lobular hyperplasia (ALH)	Lobular intraepithelial neoplasia, grade 1 (LIN1)	B3	Risk indicator and non-obligate precursor
Lobular carcinoma in situ, classic type (LCIS) Lobular carcinoma in situ with necrosis or pleomorphic	Lobular carcinoma in situ (LCIS) High-grade LCIS and Pleomorphic lobular carcinoma in situ (PLCIS)	Lobular intraepithelial neoplasia, grade 2 (LIN2) Lobular intraepithelial neoplasia, grade 3 (LIN3)	B3 B5	Risk indicator and non-obligate precursor

25 years' follow-up) have been recently published and are currently changing the management strategies of these conditions [30, 32, 33]. Moreover, some of these risk indicators have been shown to harbor molecular aberrations identical to those of the matched invasive disease and thus, they are considered breast cancer precursors [3, 34–36]. Given that only a still unidentifiable subset of these precursors will effectively progress to invasive breast cancer, they are indeed defined as non-obligate precursors of breast cancer together with in situ carcinoma.

The "predictive" system has been created for clinical management purposes. The current European Guidelines for quality assurance in breast cancer screening and diagnosis required all breast needle core biopsies to be classified according to a five-tier pathologic-based classification scheme: B1, normal; B2, benign; B3, lesion of uncertain malignant potential; B4, suspicious for malignancy; and B5, malignant [37, 38]. These categories are based only on the histological findings of the specimen and clinical management of the detected lesions has to take into account also their clinical and imaging characteristics. Given the limited specimen of a core biopsies, the B-classification does not require pathologists to give a definite diagnosis, simplifying pathological evaluation. An Italian survey on diagnostic concordance of B-classification reported a good overall interobserver agreement (mean kappa score, 0.61), with, however, lower concordance rates for B3 category [39]. Similarly, Elmore et al. reported a good overall concordance (75.3%) between US pathologists and expert consensus diagnoses, with again lower levels of agreement for atypical lesions with uncertain malignant potential [40]. The B3 category encompasses 3–10% of the histologically assessed biopsy of screening detected lesions [41]. This subgroup comprises different histopathological entities that are known to have variable risk of associated concurrent malignancy [41, 42]. Except for DCIS, all noninvasive breast lesions fall into the B3 category, including FEA, atypical ductal hyperplasia (ADH) and lobular neoplasia (LN), together with papillary lesions, radial scar, and phyllodes tumor. An improvement of the diagnostic concordance and definition of this group is warranted in order to avoid over- or undertreatment of the women with a diagnosis of B3 lesion.

The formal and traditional classification of pre-invasive lesions of the breast, re-proposes the old-fashioned concepts of ductal and lobular lesions with columnar cell changes as the third wheel. This classification has been endorsed by the last edition of WHO classification of breast tumors [9]. In particular, based on differences in quantitative and qualitative morphologic characteristics, clinical behaviors and, recently, molecular features, these entities can be categorized as follows:

- Columnar cell changes, including flat epithelia atypia (FEA)
- Lobular neoplasia, including atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), and pleomorphic lobular carcinoma in situ (PLCIS)
- Atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS)

Although it is widely adopted, this classification represents one of the most controversial topics in breast pathology. Indeed, the morphologic criteria and the nomenclature used are still debated, resulting in a high rate of diagnostic interobserver variability. In particular, the distinction between atypical hyperplasia (AH, including ALH and ADH) and in situ lesions is rather problematic. The diagnostic criteria for AH are based mainly on exclusion rather than positive features [27]. ADH and ALH are diagnosed when some features of DCIS or LCIS, respectively, are present, but others are lacking. This qualitative definition of AH has been updated to include (arbitrary) quantitative features. ADH is diagnosed when lesion foci occupy less than two separate ducts [43] or measure less than 2 mm [44]. Noninvasive lobular lesion is classified as ALH if abnormal changes involved less than 50% of acini or TDLU [9, 45, 46]. However, how to define atypical proliferations that are qualitatively identical to in situ carcinomas, but quantitatively "too small," or atypical lesions that extend over the quantitative cut-offs but fail to meet the diagnostic criteria of in situ carcinoma? In the setting of controversial boundaries between atypical lesion and in situ carcinoma, it has been suggested to abandon this terminology in favor of a three-tier framework similar to that used for intraepithelial neoplasia of cervix, vagina, and vulva. In this classification, all ductal and lobular lesions are defined as ductal intraepithelial neoplasia (DIN) and lobular intraepithelial neoplasia (LIN), respectively, including both atypical and in situ entities [47–50]. Avoiding the term "carcinoma" for lesions that are not invasive has the advantages to reduce confusion among health professionals and adverse psychological reactions among patients [50, 51]. However, this three-tier classification has not been endorsed by the 2012 edition of WHO classification since it seems to suggest a progression from low- to high-grade lesions that opposes our current, albeit limited, biological knowledge on breast cancer evolution processes [9, 50].

With all the caveats described above, and waiting for new molecular genetic techniques and biomarkers to pinpoint the basis for a revised unanimous classification system, in the next section a systematic treatise of each noninvasive breast lesions will be given following their traditional taxonomy.

11.3 Columnar Cell Lesions and Flat Epithelial Atypia

Some of the most challenging tasks in diagnostic pathology include subclassifying the spectrum of columnar cell lesions (CCLs). These common alterations of the breast that were first described by Stewart and Foote in 1945 [52], have gained renewed attention from both clinical and research standpoints, given their increased detection rates and association with a wide spectrum of malignant and benign breast lesions. High-throughput sequencing studies are currently validating the hypothesis that CCLs might constitute the "missing link" between normal TDLU and the ADH-DCIS *continuum* [53–56].

Although different classifications and names for this group of lesions have been used [57], including but not limited to flat epithelial atypia (FEA), ductal intraepithelial neoplasia (DIN) flat type, columnar cell alterations with apical snouts and secretions with atypia, enlarged lobular units with columnar alteration, atypical cystic lobules, atypical cystic ducts, and clinging carcinoma monomorphic type, the term CCL is most widely adopted. Under the umbrella of CCL stands a wide spectrum of lesions sharing the histologic hallmark of enlarged TDLUs lined by columnar epithelium. Importantly, CCLs are usually classified based on the presence of architectural and/or cytological atypia. In this respect, the classification system proposed by Schnitt and Vincent-Salomon [57], albeit strictly morphology-based, has been shown the lowest rates of interobserver variability. This system clusters CCLs into four broad groups, namely, columnar cell change (CCC), columnar cell hyperplasia (CCH), columnar cell change with atypia (A-CCC), and columnar cell hyperplasia with atypia (A-CCH). However, based on their partially overlapping molecular features, the latter two entities have been grouped by the WHO into the FEA category [9]. This consensus nomenclature has undoubtedly led more uniform identification of atypical CCLs. Regrettably, grading of CCLs remains rather problematic in terms of interobserver reproducibility.

11.3.1 Epidemiology and Clinical Features

Given that CCLs are frequently associated with microcalcifications, with the increasing frequency of mammographic screening an increased detection rate of CCLs has been observed [58]. For breast pathologists, CCC and CCH represent common findings in daily practice, being part of the spectrum of lesions that can be observed in the context of fibrocystic changes. On the other hand, A-CCC and A-CCH seem to be consistently less common. In a recent study

analyzing over 11,000 excisional breast biopsies, it has been shown that FEAs are uncommon lesions, involving less than 2.5% of benign breast biopsies [59].

11.3.2 Histological Features

CCLs are morphologically characterized by dilated acinar structures lined by a single layer of ER-positive, HER2-negative bland columnar cells with apical snouts, showing different degrees of cytological atypia. As described in the Schnitt and Vincent-Salomon classification [57], these lesions are made up by dilated enlarged TDLUs lined by columnar-shaped cells that can be arranged in mono/bi-stratified (CCC and A-CCC) or pluristratified (CCH and A-CCH) epithelia. The columnar cells characteristically show uniform ovoid-to-elongated nuclei with no or inconspicuous nucleoli. In A-CCC and A-CCH, the cytological atypia can be identified by the presence of rounder nuclei that might also show irregular borders, prominent nucleoli, and increased nuclear/cytoplasmic ratio (Fig. 11.1a and b). Not uncommonly, atypical columnar cells are irregularly oriented along the basement membrane of the TDLU. Mitotic figures, although exceptional, can also be observed, while complex architectural patterns are considered characteristic of ADH and low-grade DCIS and should not be present in CCLs. Apical cytoplasmic blebs or snouts are not uncommon at the luminal interface in CCLs. Intraluminal periodic acid–Schiff (PAS)-positive secretions and variable clusters of amorphous-to-pleomorphic tiny specks of calcium (microcalcifications) are frequent, representing the only mammographic signal of the possible presence of CCLs. Of note, CCLs cannot be identified clinically or on macroscopic examination, their diagnosis being currently histopathology-based and, they are often associated with other alterations of the breast in the context of fibrocystic changes (Fig. 11.1a and b).

All categories of columnar cell lesions typically show diffuse and intense ER expression and low proliferative rate (Ki-67) [57].

11.3.3 Molecular Pathology

The genetics that underpins CCLs is not at all fully clarified. However, recent molecular studies have provided evidences that CCLs, in particular FEAs, are clonal and possess neoplastic features, such as the presence of recurrent copy-number alterations, (CNAs), including losses of 16q and chromosome X and gains of 15q, 16p, 17q, and 19q, as defined by CGH [5, 53–56]. Allelic imbalances have been detected in CCLs and most commonly target chromosomes 3p, 9q, 10q, 11q, 16q, 17p, and 17q [55, 56]. Importantly, the degree of genetic instability found in CCLs seems to reflect the degree of atypia found in different types of CCLs [53, 55]. Furthermore, their frequent association with ADH/low-grade DCIS [18, 60, 61], their identical immunoprofiles [19], and the partially overlapping molecular alterations between CCLs and matched ADH [53], allowed some authors to identify CCLs as bona fide non-obligate precursor to invasive breast cancer. At present, CCLs are considered as a part of the so-called low-grade breast neoplasia family, constituting the earliest histologically identifiable breast lesion linked to cancer progression [3, 19, 53]. Intriguingly, specific miRNA signatures (e.g., miR-132 overexpression) have been recently identified in CCHs and

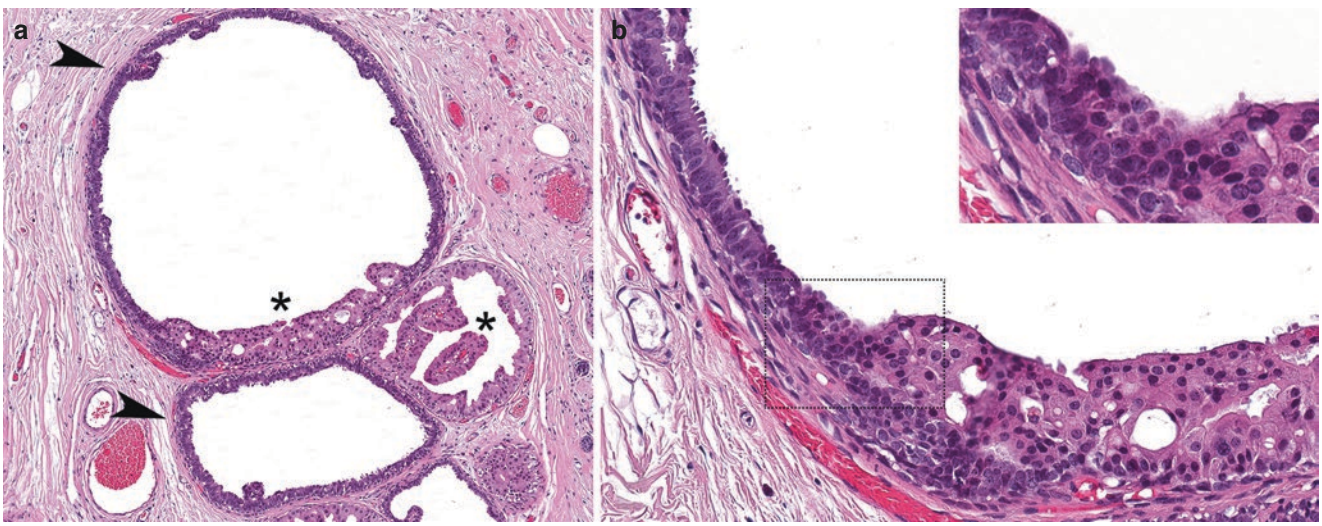

Fig. 11.1 Columnar cell lesion (arrowhead) in the context of fibrocystic changes with foci of apocrine hyperplasia (star). At higher magnification, cells with intermediate cytological features can be observed in the transition areas. ((**a**), hematoxylin and eosin, original magnification 50; (**b**), hematoxylin and eosin, original magnification 200×)

their surrounding stromal compartment, suggesting that epithelial and stromal miRNA changes may represent very early important changes in breast cancer progression [62].

11.3.4 Clinical Implications and Subsequent Risk of Invasive Breast Cancer

Despite the emerging molecular information regarding their neoplastic nature, the association between CCLs and subsequent breast cancer risk remains undefined, with many retrospective studies showing heterogeneous results. The risk of developing breast cancer in patients with a diagnosis of FEA have been estimated at one to two times higher than those without FEA [57, 63–65]. However, the recently published Mayo Cohort Study have shown that a diagnosis of pure FEA seems not convey independent risk of breast cancer [59]. Since some CCLs diagnosed in breast core biopsies are associated with more advanced lesions in the remaining breast, this poses difficulties for the optimal management when found on core needle biopsies. CCC or CCH are regarded as benign (B2 on core biopsy), and there is no need of any additional assessment [37]. Importantly, the presence of FEA foci in a core biopsy is reported to be associated with a high risk of DCIS/invasive carcinoma in subsequent surgical excision specimens (B3 lesion) with an upgrade rate after a vacuum-assisted breast biopsy (VABB) diagnosis of pure FEA that ranges from 0 to 20% [66, 67]. In the recently published First International Consensus Conference on lesions of uncertain malignant potential in the breast (B3 lesions), among 177 cases of FEA that had subsequent therapeutic open surgical excision following VAB diagnosis, the upgrade rate to invasive malignancy was 9% [68]. These data have been provided to support the detection of atypical CCLs in a core biopsy as sensors for monitoring patients with higher risk to develop advanced lesions, therefore justifying their surgical excision [68].

As the natural history of CCLs is not yet well known, this generates difficulties for further clinical management. At present, the optimal management of CCLs patients remains to be determined. However, two major issues have to be acknowledged in setting up diagnostic, treatment, and follow-up strategies while dealing with CCLs. First, clinicians, radiologists, pathologists, and research scientists should avoid any synecdochic approach in the interpretation of breast biopsies with CCLs. Indeed, it is widely recognized that breast core biopsy samples are not necessarily representative of the entire lesion, as CCLs might be a part of an even more complex mosaic of premalignant alterations (e.g. ADH, DCIS, and tubular carcinoma). The second point involves the long-standing topic on the optimal risk-benefit ratio to allow (and recommend) breast surgery in this era of precision medicine. Specifically, despite the recent gains in the diagnosis and treatment of these patients, it is currently extremely difficult to anticipate which of the entities belonging to each CCL category are associated with an increased long-term risk of related invasive cancer. On the other hand, other more aggressive lesions can be present at the periphery of the CCL and might not be straightforward to be sampled based on the intrinsic limitations of mammographic techniques. For CCL without atypia, more studies with a long-term follow-up coupled with high-throughput molecular investigations are warranted, but so far, surgical excision biopsy does not seem to be necessary.

11.4 Atypical Lobular Hyperplasia and Lobular Carcinoma In Situ

Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) are considered part of a spectrum of noninvasive breast lesions often referred to as lobular neoplasia (LN). The term LCIS was first coined in by Foote and Stewart to indicate noninvasive lesions of the breast with cytological resemblance to invasive lobular carcinoma (ILC) [69]. Less well-developed proliferative lesions with morphologic features similar to those of LCIS but associated with a lower risk of breast cancer development were subsequently named ALH [70]. In the late 1970s, Haagensen introduced the umbrella term LN that soon became widely used both in clinical and academic settings [71]. This definition has the undeniable advantage to overcome the problematic distinction between ALH and LCIS but, regrettably, is not able to capture the diverse clinical implications of these lesions. Noninvasive lobular neoplasia is also defined as lobular intraepithelial neoplasia (LIN), with the three-tier grading system proposed by Tavassoli that classified ALH as LIN1, classic LCIS as LIN2, and high-grade and pleomorphic LCIS as LIN3 [48]. ALH and LCIS have been historically considered as risk indicators for subsequent development of IBC, but there are growing observational and molecular evidences to suggest that at least a subset of them are true non-obligate precursors [46, 72–76]. At present, the classification of noninvasive lobular lesions, together with their clinical and biological implications and consequently the appropriate management of women with a diagnosis of ALH or LCIS, are still a multidisciplinary conundrum.

11.4.1 Epidemiology and Clinical Features

Noninvasive lobular lesions are most frequently observed in pre- and perimenopausal women [71, 77]. Since these lesions have no specific clinical and mammographic features, their diagnosis is usually an incidental finding [69, 71]. Therefore, it is difficult to estimate their real prevalence. Large cohort retrospective studies suggest that

LCIS is diagnosed in approximately 0.5–4% of women with otherwise benign breast biopsies [71, 78–80]. It is even more challenging to establish the overall ALH incidence given that this lesion is frequently combined with either LCIS or ADH. In a recent series from the Johns Hopkins Institution, among 10,024 breast core biopsies performed, only 117 (0.1%) cases had a diagnosis of pure ALH [81]. Although remaining a relatively uncommon finding, the incidence of LCIS showed an increased incidence among population-based data of Surveillance, Epidemiology, and End Results (SEER), growing from 2 to 2.75% in 2000 and 2009, respectively [82]. Multifocality and bilaterality are key characteristics lobular neoplasia, as multifocal and multicentric disease is detected in over 50% of cases, while nearly 30% of patients have bilateral lesions at diagnosis [83, 84].

11.4.2 Histological Features

The histological features of ALH and LCIS have long been well established [9, 69, 71]. These lesions are characterized by monomorphic proliferation of small round-to-polygonal discohesive cells that distend the acini with maintenance of the lobular architecture. The neoplastic cells have usually scant clear cytoplasm with high nuclear-cytoplasmic ratio but intracytoplasmic vacuoles composed of darkly staining dots (formerly known as magenta bodies), can often be found (Fig. 11.2a). The abnormal cells frequently show a pagetoid spread, with a characteristic upward diffusion along the ducts, between the normal epithelium and basement membrane.

The distinction between ALH and LCIS is mainly based on quantitative criteria. While the abnormal cells in ALH only partially fills and distends the acini, a diagnosis of LCIS is allowed when the acini are completely filled, with no more visible lumina, and more than 50% of TDLU is involved by the lesion [9, 45]. Regrettably, adopting the above criteria in daily practice is by far problematic, resulting in extraordinarily high rates of inter- and intra-observer variability [85]. Beside the mainstream histological features, additional morphologic details have been used to subclassify LCIS in several variants. The prototypical cells of LCIS with inconspicuous cytoplasm and small bland nuclei with a size similar (1.5×) to that of lymphocytes have been defined as "type A" cells. Conversely, "type B" cells have larger clear cytoplasm compared to that of type A, as well as larger nuclei with mild-to-moderate atypia [86]. Although this distinction has been demonstrated to have only a descriptive meaning, a subset of type B LCIS can display a highly aggressive clinical behavior, as compared to classic variants [87]. Indeed, more than two decades ago, Eusebi, Magalhaes, and Azzopardi described an aggressive variant of LCIS that was named pleomorphic lobular carcinoma (PLCIS) [88], given the eccentric large pleomorphic nuclei (4× bigger than a lymphocyte nucleus), prominent nucleoli and peculiar large eosinophilic granular cytoplasm harbored by the neoplastic cells (Fig. 11.2b). In this LCIS type, necrosis and microcalcifications are frequent. Historically, it has been suggested that PLCIS should be treated following the recommendations of DCIS; however, definitive data regarding their natural history are still missing [85, 89]. Interestingly, recent genomic studies have provided evidences that PLCIS is more closely related to LCIS rather than DCIS [90].

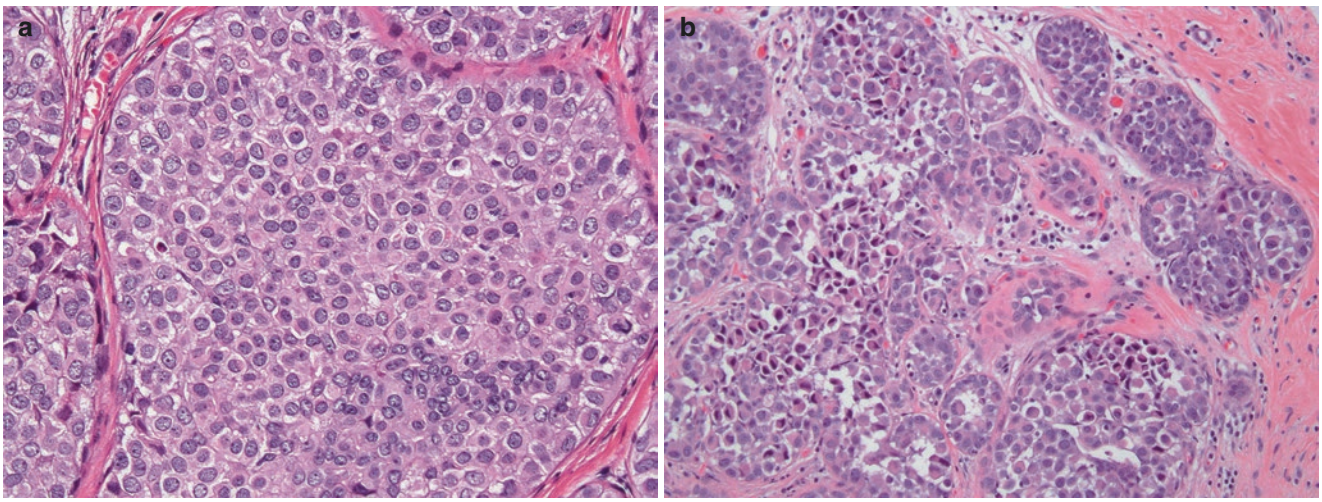

Fig. 11.2 Histological features of lobular carcinoma in situ (LCIS). (**a**) Monomorphic proliferation of polygonal discohesive cells with clear cytoplasm that distends the acini with maintenance of the lobular architecture. (**b**) In situ lesion with lobular phenotype, showing eccentric large pleomorphic nuclei, conspicuous nucleoli and large eosinophilic granular cytoplasm, consistent pleomorphic lobular carcinoma in situ (PLCIS). Hematoxylin and eosin, original magnification 200×

Conventional lobular neoplasia typically shows an immunoprofile similar to that of the luminal A invasive counterpart, such as strong and diffuse expression of ER and progesterone receptor (PR), no HER2 overexpression and low proliferation index (Ki-67) [85]. Importantly, the rule exception is represented by the high-grade LCIS and PLCIS, which are characterized by a higher Ki-67 index, low or absence of ER/PR expression, and possible HER2 overexpression/amplification [85]. Instead, loss of membranous expression of E-cadherin connotes the entire spectrum of lobular neoplasia, and it is considered (and often abused) a cornerstone diagnostic biomarker for both in situ and invasive lobular diseases. E-cadherin is a transmembrane molecule found in cell adherens junctions. Dysfunctional protein leads to loss of cell adhesion, representing the molecular substrate of the characteristic discohesive architecture of the lobular lesions [9, 86, 90–92]. However, aberrant E-cadherin expression may be observed in a proportion of LCIS; therefore, this diagnosis cannot be entirely ruled out in the presence of the clear-cut lobular morphology in E-cadherin positive cases [92]. In case of ambiguous histological appearance, evaluation of p120 catenin expression can be performed in order to confirm the diagnosis. This protein is a membrane-located cell adhesion molecule that accumulate in the cytoplasm of cells with absent or dysfunctional E-cadherin [92].

Given our long-term dealing with LN histologic features, the diagnosis is usually straightforward. However, in a subset of cases showing hybrid histological features, the distinction between classical LCIS and low-grade solid DCIS can be challenging, and it is not always achievable [85]. Furthermore, the cytological features of PLCIS, together with the presence of necrosis and calcification, can lead to the misdiagnosis of high-grade DCIS [85]. In these tricky cases, careful evaluation of morphological characteristic of neoplastic cells, the use of specific biomarkers such as E-cadherin, and asking for second opinion can assist in the correct diagnosis and ultimately lead to the proper clinical management of these patients.

11.4.3 Molecular Pathology

The molecular landscape of LNs is distinctively characterized by loss of expression and/or inactivation of E-cadherin. The inactivation/dysregulation of E-cadherin results from a combination of genetic, epigenetic, and transcriptional alterations [3, 93–96]. Loss of E-cadherin (*CDH1*) gene locus at 16q22 is frequently observed in LCIS as well as inactivating somatic mutations of *CDH1* [76]. Additional recurrent molecular alterations are those that pertain to the so-called low-grade estrogen receptor (ER)-positive breast neoplasia family, including the expression of ER and ER-related genes and the aforementioned deletions of 16q, as well as gains of 1q and 16p [97, 98]. In a recent survey performed on 34 LCIS samples from Memorial Sloan Kettering Cancer Center (MSKCC) using a targeted capture massively parallel sequencing platform, *CDH1* mutations have been detected in 56% of LCIS [76]. Interestingly, then vast majority of *CDH1* alterations were coupled with loss of heterozygosity (LOH) of the gene. Furthermore, *PIK3CA* has been shown to represent second more frequently mutated gene in LCIS. Intriguingly, the authors of this study detected a similar repertoire of somatic mutations in LCIS and paired invasive lobular carcinoma (ILC), providing the molecular evidence that at least some LCIS are non-obligate precursors of IBC. Previous comparative genomic hybridization studies have also shown that LCIS and ILC display similar recurrent copy-number profiles [99]. Formal clonal relationship between LCIS and ILC have been recently demonstrated also by SNP array and whole-exome sequencing analyses [75, 100].

Despite the relatively homogeneous histological and genomic feature of LCIS, heterogeneity has been described at transcriptomic level [101]. In particular, two molecular subtypes of LCIS have been identified based on differentially expressed genes, including proliferation genes and genes of cancer-related pathways (e.g., actin cytoskeleton, apoptosis, p53 signaling, TGF beta signaling, and Wnt signaling). Although the two molecular clusters displayed significantly differences in Ki67 expression levels, no significant correlations have been found between these subtypes and their clinicopathologic features [101].

Finally, the few genomic analyses performed on PLCIS confirmed that these lesions belong to the LN spectrum [90, 102–104]. Indeed, PLCIS have been shown to harbor the hallmark copy-number changes of classic variants of LCIS (i.e., loss of 16q and gain of 1q and 16p) but also display higher rates of genomic instability, with frequent amplification of *MYC* and *HER2*. This molecular scenario may represent the genomic substrate of the clinical aggressiveness frequently observed in PLCIS.

11.4.4 Clinical Implications and Subsequent Risk of Invasive Breast Cancer

LCIS has long been regarded as a risk indicator for subsequent development of IBC. On the other hand, when the term LCIS was coined this group of lesions was dogmatically defined as the pre-invasive step of ILC. Early observational studies [83, 105, 106], however, went against this concept, observing that (1) the risk of IBC conferred by LCIS is lower when compared to the bona fide real precursor, (2) IBC may develop after a diagnosis of LCIS either in ipsilateral and contralateral breast,

and (3) either of lobular and ductal histotypes. Overall, it has been estimated that a diagnosis of LCIS confers a risk of 1–2% per year for subsequent ILC, with a relative risk of 8–10 [32, 71, 78, 105]. Furthermore, the long-term cumulative risk ranges from 11 to 26% at 15 years [32, 71, 77, 105, 107]. On the other hand, women with ALH have a relative risk for later IBC of 4 with a long-term cumulative risk of 27–30% after 25 years [33]. Given these relatively high cumulative risks, application of standardized pathological criteria of ALH is pivotal for reducing the high inter- and intra-observer variability of these diagnoses, especially in small diagnostic specimens. Taken together, the gap between the relative risks of ALH and LCIS underlines the fundamental role of an accurate histopathological distinction between these two entities, discouraging the use of diagnostic ellipsis such as LN. According to the European Guidelines for quality assurance in breast cancer screening and diagnosis [37], LCIS and ALH are both classified as B3 lesions, given the risk of concurrent malignancies with a rates of upgrades after surgical excision that ranges from 0 to 67% [108, 109]. However, if there is concordance between radiologic and pathological findings, the upgrade rate for ALH drops to 0–6% and routine surgical excision is not mandatory in these cases [108, 110].

Back to the initial identification of LCIS and ALH as discrete clinicopathologic lesions, pathologists attempted to detect the morphological abnormalities not only within the lesions themselves but also in their surrounding tissue in order to stratify the risk of the patients for subsequent invasive cancer. In the National Surgical Adjuvant Breast Project (NSABP) Protocol B-17 and the National Surgical Adjuvant Breast and Bowel Project studies, 19 histopathological parameters have been assessed as potential predictors for IBC occurrence [111, 112]. These features included the number of involved lobules, duct extension, type of LCIS (using a grading system similar to that proposed by Tavassoli), intralesional calcification, nuclear grade, cell size, cell variants (e.g., signet ring and histiocytoid), mitotic rate and lymphocytic infiltrate. The results of this study showed that only grade 2 and grade 3 LCIS are significantly associated with increased short- and long-term risks of developing IBC. In the recently published 29-year longitudinal series of MSKCC that include 1032 women with a diagnosis of LCIS undergoing surveillance, among the numerous pathological variables assessed in a case-control analysis, only the disease volume (defined as the ratio of slides with LCIS to total number of slides reviewed), was found to be associated with IBC development [32].

Although ALH and LCIS are indubitably risk indicators of later IBC, current clinico-epidemiological and high-throughput sequencing data support the contention that at least a subset of these lesions are also non-obligate precursors of IBC. ALH and LCIS confer a bilateral risk of IBC that can be either of IDC and ILC; however, the majority of studies reported a higher incidence of ipsilateral ILC [46, 86, 112]. Furthermore, recent molecular studies have demonstrated the presence of identical genetic aberrations in LCIS and matched ILC, confirming that these two lesions are clonally related [75, 76, 99, 100]. 75 years later LCIS was first described, we are back to the original idea about LCIS nature as both risk indicator and non-obligate precursor. Regrettably, we are still no able to identify which of these precursors will evolve to IBC, since the biology of this progression remain poorly understood. For these reasons, the clinical management of ALH and LCIS continues to be a challenge with a plethora of possible management options, ranging from simple observation to radical surgical approaches.

11.5 Atypical Ductal Hyperplasia and Ductal Carcinoma In Situ

Atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) are part of a wide group of premalignant and pre-invasive breast lesions that are characterized by proliferation of neoplastic epithelial cells confined within the lumen of TDLUs with preservation of intact basement membrane [9]. DCIS is a well-established non-obligate precursor of invasive breast cancers (IBCs). The observation of spatial (i.e., DCIS adjacent to IBC) and temporal (i.e., IBC developed after a diagnosis of DCIS) proximity between these two entities, the usually concordant nuclear grade and immunophenotype of adjacent DCIS and IBC as their genetic similarity and clonal relationship represent the plethora of evidence of their clinico-biological continuity. However, only a subset of DCIS will progress to IBC (i.e., non-obligate precursor). The identification of the biology underpinning of the DCIS to IBC progression and ultimately the development of biomarkers able to predict which patients will progress are the main clinical, pathological, and molecular challenges (and tasks) posed by DCIS.

ADH is considered both a risk indicator and non-obligate precursor, displaying morphological and genetic features similar to those of low-grade DCIS but a lower risk of breast cancer development. Differentiation of ADH from low-grade DCIS is anything but simple. The histologic criteria for diagnosing of ADH have been known for a long time, but they still represent one of the most controversial issues in breast pathology. The DIN classification is able to highlight the continuum between ADH (DIN1b) and low-grade DCIS (DIN1c). However, this terminology suggests a progression from low-grade to high-grade DCIS (DIN2/3) that oversimplify the "tangle" model of the progression from DCIS to IBC.

11.5.1 Epidemiology and Clinical Features

ADH is a relatively uncommon condition that represent the diagnostic finding in up to 4% of symptomatic benign biopsies [113, 114]. However, the epidemiological data on ADH continue to suffer from the variable interobserver application of diagnostic criteria. Conversely, the incidence of DCIS underwent a precipitous increase following the spreading trend of mammographic screening program that peaked in 2000 and stabilized at lower increase rate after 2005 [1, 115–117]. According to the American Cancer Society, Surveillance Research, 60,290 women were diagnosed with DCIS in 2015, accounting for 17% of all breast cancer diagnosis and of 83% of in situ carcinoma [1, 118]. The majority of DCIS was detected in postmenopausal women with peaks at ages 70–79 [118]. Risk factors for the development of DCIS are similar to those of IBC supporting the evidence of their etiologic relationship [119–121].

Microcalcifications represent the characteristic mammographic sign of DCIS, although masses or areas of architectural distortion can also be found [118, 122].

11.5.2 Histological Features

"*The histologic criteria for diagnosing atypical lesions rests heavily on definitions of histologic features of carcinoma in situ. We demand that all features of carcinoma in situ […] be uniformly present throughout two separate spaces before ductal carcinoma in situ (DCIS) is diagnosed". Anything less will occasion a diagnosis of the corresponding atypical lesion if some of the features of carcinoma* in situ *are present*" (Fig. 11.3). These are the diagnostic criteria for the diagnosis of ADH that were established by David Page and colleagues in 1985 [27]. Indeed, the histopathological features of ADH are essentially those of low-grade DCIS, except for the extent of the lesion. Differentiation of ADH from low-grade DCIS is based on a single criterion that, albeit arbitrary, is rather simple and undeniably pragmatic: if the lesion involves less than two membrane-bound spaces [43] or measures less than 2 mm in greatest dimensions [44], it should be classified as ADH; if not, as DCIS. Importantly, the WHO recommends to perform a diagnosis of ADH only if a diagnosis of low-grade DCIS has been seriously considered [9]. A long-term plethora of studies have reported on the difficulty in achieving acceptable levels of concordance among pathologists in diagnosis of ADH (and other borderline lesions of the breast) [40, 114, 123–127]. Since the correct identification of ADH is an essential step for the proper clinical management of the patients, revision of current criteria with the integration of more reproducible histological and molecular biomarkers should be a future effort for the pathologists.

The term DCIS does not identify a single entity but a spectrum of noninvasive lesions with heterogeneous clinical, morphologic, and molecular features. DCIS are primarily classified in low, intermediate or high-grade based on nuclear features and mitotic activity [128]. Nuclear grade has been demonstrated to be of clinical relevance as predictor of local recurrence [129–131]. The DIN system mirrors this classification considering three categories, DIN1c, DIN2, and DIN3, with increasing nuclear grade [48, 49, 128]. In the routine pathological assessment of DCIS, it is recommended to report also the size of the lesion, the architectural pattern/s, the presence of necrosis, in particular the central confluent necrosis (i.e., comedo

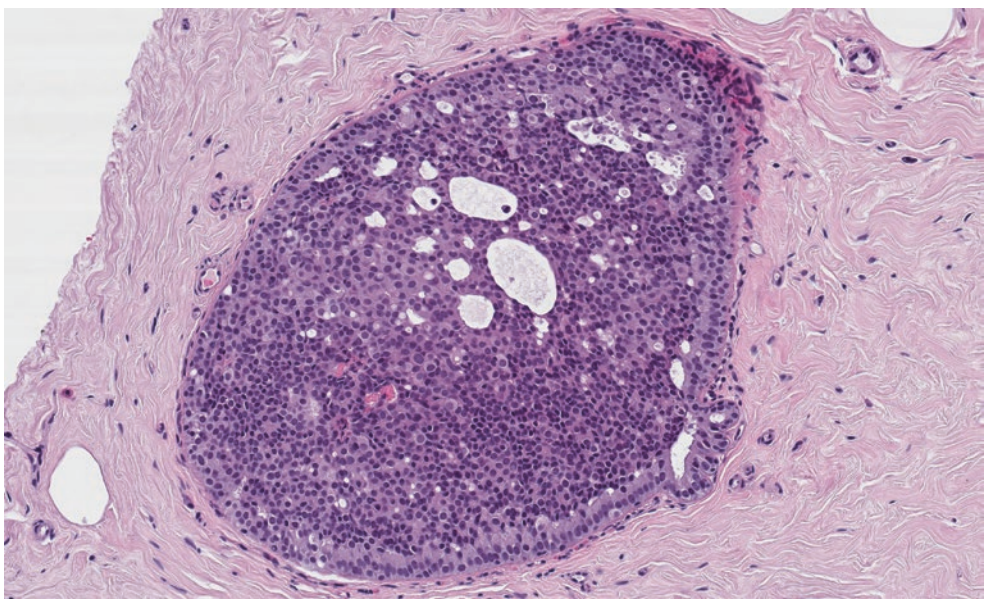

Fig. 11.3 Histological features of atypical ductal hyperplasia (ADH) from a core needle biopsy. In this paradigmatic example of ADH, bland cells are contained within the duct, forming rigid cell "bridges" across the duct space (hematoxylin and eosin, original magnification 100×)

necrosis), the presence of microcalcifications for imaging-pathology correlation, and the distance from excision margins [128, 132, 133].

Low-grade DCIS is characterized by a proliferation of monomorphic cells with slightly enlarge nuclear size, finely dispersed chromatin, inconspicuous nucleoli, and rare mitoses that display variable often intermingled architectural patterns, including cribriform (with neoformed smooth outlined lumina), micropapillary, and solid patterns. Diffuse expression of ER and PR and lack of HER2 overexpression are constantly seen in low-grade DCIS. Apart from differential diagnosis with ADH, low-grade DCIS has to be distinguished from ductal hyperplastic, non-atypical, lesions. The key words for this differential diagnosis are cell monomorphism vs. polymorphism, architectural regularity vs. disorder and diffuse strong vs. modulated ER expression for DCIS and hyperplastic lesions, respectively [9, 128, 134].

Intermediate-grade DCIS display nuclear features (and morphologic characteristics) in between low- and high-grade DCIS [128, 134].

Pleomorphic cells with large nuclear size with prominent nucleoli and mitosis connote high-grade DCIS. Solid or flat (clinging) pattern of growth and comedo necrosis are often seen in this lesion (Fig. 11.4) [9, 128, 134].

The presence of invasive or microinvasive breast cancer has to be excluded before establishing any definite diagnosis of DCIS. The presence of IBC can be usually ruled out based on morphologic assessment. However, in particular cases, especially in small specimens, immunohistochemical evaluation of myoepithelial cell markers (e.g., p63, calponin) that are retained in DCIS and absent in IBC can be useful diagnostic tools [9].

11.5.3 Molecular Pathology

The morphologic variability of DCIS is mirrored by its molecular heterogeneity. ADH and low-grade DCIS display similar immunophenotypes and genetic aberrations. Both ADH and low-grade DCIS are characterized by strong expression of ER and a nearly-identical pattern of recurrent genetic aberration including, losses of 16q, gains of 1q and 16p, that are commonly found in lesions of low-grade ER-positive breast neoplasia family [3].

It is more difficult to identify common molecular denominators in high-grade DCIS since they display heterogeneous immunophenotypic and genetic features. However, the recurrent genetic aberrations detected in low-grade lesions are uncommonly found in high-grade DCIS, suggesting nuclear classification is able to properly identified different entities that mostly evolve through distinct pathways [3]. Moreover, gene expression profile analyses have identified differentially expressed gene in low- and high-grade DCIS [16, 135]. Balleine et al. conceived a "molecular grading" model of DCIS that identified low- and high-grade lesions based on gene expression and comparative genomic hybridization data. In this study, the combination of routine histopathological features of DCIS, including nuclear grade and Ki67 score, was able to predict the "molecular grading" in the 96% of the cases [16]. As for the majority of histopathological "intermediates," no distinct molecular characteristics have been identified in DCIS of intermediate nuclear grade that have been shown to split between molecular low-grade and high-grade group [16, 17].

Gene expression profile studies have also demonstrated that the whole spectrum of the intrinsic molecular subtypes

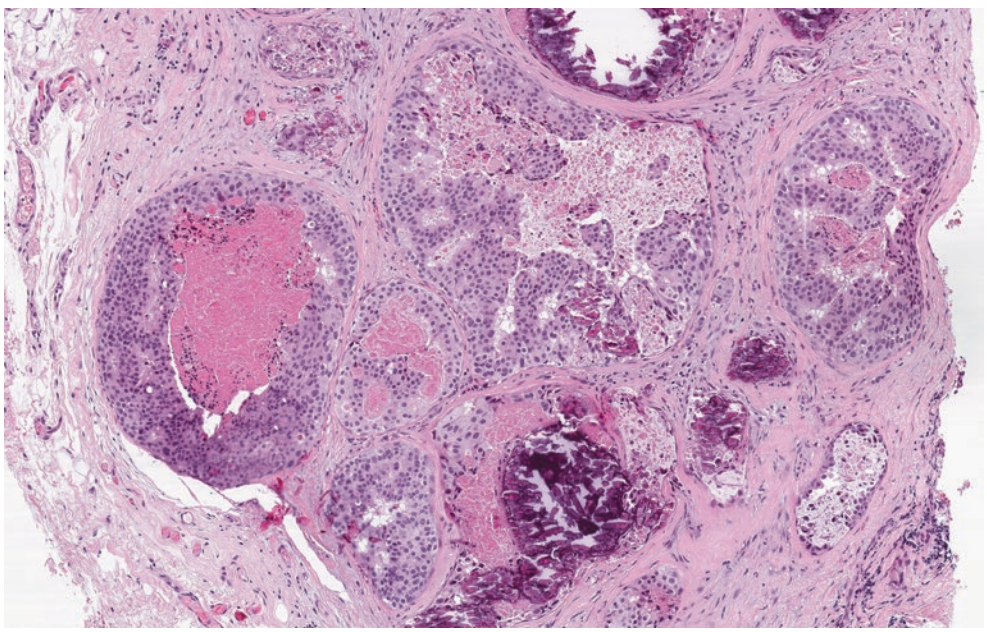

Fig. 11.4 Histological features of high-grade ductal carcinoma in situ (DCIS) from a core needle biopsy. In this intraductal carcinoma of the breast, comedo necrosis can be observed in the central luminal area, as well as peripheral calcifications (hematoxylin and eosin, original magnification 50×)

of IBC are encountered in DCIS [136–140], albeit with a higher frequencies of luminal B and HER2 positive neoplasia [136]. Far away from being completed, the current molecular knowledge on DCIS suggests that in routine practice, a good morphology-based nuclear classification and the evaluation of hormonal receptor status, HER2 expression, and Ki-67 proliferation index by immunohistochemistry can classify DCIS in distinct molecular and ultimately clinical entities. Indeed, beside classification purposes, the great effort of molecular studies on DCIS have been directed toward the identification of predictive biomarkers for the risk of progression and the biological mechanisms of DCIS-IBC evolution.

11.5.4 Clinical Implications and Subsequent Risk of Invasive Breast Cancer

While morphological and molecular data suggest that ADH, DCIS, and IBC are likely to be phylogenetically related, these diagnoses have substantially different clinical repercussions. Indeed, women with ADH have approximately three- to fivefold increased risk of developing breast cancer [27, 141, 142], either DCIS and IBC, while DCIS confers substantially higher risk of developing IDC (eight- to tenfold increased risk) [3, 143] with an absolute risk of progression to IBC at 10 years ranging from 20 to 53% [143–147]. Though the relative risk for ADH is low, absolute risk data have shown a cumulative risk for either DCIS or IBC of 30% at 25 years of follow-up [30], and a 5.7% 10-year cumulative risk of IBC after a diagnosis of ADH [148]. Moreover, as a B3-defined lesion, a diagnosis of ADH does not entail only a risk of later IBC development but also the risk of concurrent associated malignancy when detected in small bioptic specimens. Indeed, the intrinsic limitation of all presurgical diagnostic/screening procedures is that pathologists can reason on the areas surrounding the sampled lesions only in terms of probabilistic logic. Therefore, any categorical syllogism should be avoided in the diagnosis of ADH given that a surgical excision may easily upgrade the diagnosis from a B3 to a B5, with a risk of underestimation for VAB biopsies that ranges from 0 to 65% [68, 149–151]. This could represent, at least in part, the reason underpinning the historical and current conduct of removing ADH.

Given the high risk of recurrent/progression subtended by DCIS, the vast majority of patients are still subjected to surgical treatment followed by radiation and/or prophylactic systemic therapies (e.g., tamoxifen) [152]. However, not all DCIS patient will recur or progress to invasive cancer. The critical issue and need for clinician and pathologists is to better stratify patients with DCIS enabling appropriate treatment selection. Nowadays, the potential selection of low-risk patients remains dependent upon combination of traditional clinical and histopathological features as those include in validated prognostic tools such as the Van Nuys Prognostic Index (VNPI) [153, 154] and the predictive nomogram from MSKCC. A modified Oncotype DX (Genomic Health, Redwood City, CA, USA) recurrence score for IBC has been implemented for DCIS [155]. This assay is based on the expression of seven cancer-related genes and five reference genes to generate a score that give a probability of DCIS recurrence at 10 years. Although it has been validated in large prospective cohorts [156, 157], it is not currently used in clinical practice since even the low-risk group had an 11% risk of any recurrence, which is not enough low to consider to spare radiotherapy in these patients [158, 159]. Among the routinely assessed biological markers (ER, PR, HER2 and Ki-67), none is strictly recommended in clinical practice. ER-positive DCIS have a lower risk of recurrence as compared to HER2-positive or triple negative lesions [130]. Risk assessment in patients with HER2-positive DCIS have suggested that HER2 overexpression was only associated with increased risk of noninvasive recurrence [160–163].

Although there have been numerous efforts to develop molecular biomarkers to predict which patients are likely to develop invasive disease following a diagnosis of DCIS, there is currently no test with demonstrated clinical utility to identify this population. It is possible that current investigation into the biological determinants of the phenomenon of progression from in situ to invasive disease will bring more useful molecular markers to predict accurately the progression from DCIS to IBC.

11.5.5 Modelling Progression from DCIS to Invasive Breast Cancer

There are many fascinating theories of progression from DCIS to IBC, most of which fall broadly into two categories. According to the "genomic theory," invasiveness is an acquired behavior that relies on specific genetic aberrations occurring in the neoplastic cells. To support this hypothesis, several models relying on Darwinian evolution principles have been recently provided [164]. Such studies focused mainly on the in-depth genetic analyses of synchronous ipsilateral DCIS and IBCs. When analyzed as a group, similar pattern of genetic aberrations [165–169] and similar gene expression profiles [140, 170] have been found in DCIS and synchronous IBC. However, recent studies based on pairwise comparisons between DCIS and IBC have revealed the existence of significant genetic differences, which are distinct from patient to patient, confirming previous observations that DCIS and IBC are lesions harboring only a few recurrent somatic molecular alterations [171, 172]. Interestingly, molecular analyses taking into account intra-tumor heterogeneity of both DCIS and IBC occurring synchronously in

close proximity, revealing that that these neoplasms show extensive intralesional genetic heterogeneity [171, 172]. Based on these data, DCIS may be depicted as a mosaic of tumor cells harboring both founder genetic aberrations (i.e., clonally detected in the vsast majority of tumor cells) as well as private mutations (i.e., present only in a subpopulation of cells). Tumor progression may occur by means of selection of specific genetic aberrations (clonal selection), which are different from patient to patient, suggesting that transition from DCIS to IBC may represent a convergent phenotype driven by Darwinian selection [164].

On the other hand, the identification of specific characteristics in the stroma surrounding DCIS and its tumor microenvironment, lead to the "non-genomic theory," where the progression from DCIS to IBC is not necessarily dependent on the acquisition of additional genetic alterations. To this end, several evidences have been provided to explain the substantial lack of genomic and transcriptomic differences between DCIS and IBC. Besides forming a physical barrier, myoepithelial cells also actively secrete in the extracellular matrix several components and protease inhibitors. In particular, recent observations support the hypothesis that the remodeling of the DCIS extracellular matrix, under certain conditions, may favor the progression to invasive disease [173–176]. Additionally, gene expression studies showed that substantial changes may occur during progression from DCIS to IBC in various cells composing the tumor microenvironment, such as fibroblasts, myoepithelial cells, and leukocytes [177–179]. However, the biologic processes underpinning such differences in gene expression remain unclear. There are several evidences to suggest that the normal myoepithelium may act as tumor suppressor on DCIS [180–182]. Indeed, both myoepithelial cells and fibroblasts surrounding the in situ lesions have been shown to harbor a rather simple genome, with the substantial absence of clonal genetic aberrations [183]. Consequently, it has been suggested that epigenetic alterations in the stroma may be involved in the progression from DCIS to IBC through the alteration of the protective effect of the normal myoepithelium [184–186]. Clinical evidence have underlined the important predictive and prognostic role of the host immune response in breast cancer [187–189]. Although few recent studies have reported on the characteristic of the immune milieu of DCIS [190–192], the role of immune microenvironment in the progression from DCIS to IBC have yet to be elucidated.

Both the genomic and the non-genomic standard models have important deficiencies, given that, alone, they are undoubtedly not able to embrace the extraordinary complexity that underpins the natural history of IBC. A paradigm shift toward new frameworks encompassing multiple systems of breast cancer genomics, epigenomics, and transcriptomics is needed. This would allow the implementation in the field of breast cancer of a comprehensive "theory of everything" in which IBCs, pre-invasive alterations, and non-obligate precursors are stratified using integrative models at an individualized level.

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Prognostic and Predictive Role of Genetic Signatures

12

Giancarlo Pruneri and Francesca Boggio

12.1 Gene Expression Reveals Inter-tumor Heterogeneity: BC Intrinsic Subtypes

A robust body of evidence, initiated by the seminal studies of Dr. Perou's group at the dawn of the new millennium [1, 2] and repeatedly confirmed over the following decade, has convincingly demonstrated that breast cancer (BC) is a heterogeneous disease further classifiable in at least four molecular intrinsic subtypes (luminal A and B, HER2 enriched, basal-like, and normal breast), based on hierarchical clustering of the "intrinsic genes" (i.e., genes with minimal variation within a tumor sample, but maximal variation between different patients) expression profile. These studies were originally based on genome-wide gene expression profiling from microarray datasets and progressed to a PCR-based test with a list of 50 genes (the PAM50 gene signature) [3, 4] (Fig. 12.1).

Recently, the NanoString nCounter^{DX} Analysis System has been shown to provide more precise and accurate measures of mRNA expression levels in formalin-fixed, paraffin-embedded (FFPE) tissue when compared to PCR [5]. Actually, PCR-based assays require excessive optimization from archival FFPE samples, thus introducing amplification biases, due to high mRNA fragmentation and cross-links to protein upon fixation. Luminal A and B subtypes are largely distinguished by the expression of two main biological processes: proliferation-/cell cycle-related and luminal/hormone-regulated pathways. Compared to luminal A, luminal B tumors are characterized by higher expression of proliferation-/cell cycle-related genes or proteins (e.g., Ki-67) and lower expression of several luminal-related genes or proteins such as the progesterone receptor (PgR) and FOXA1, while estrogen receptor (ER) is expressed at similar levels in the two subtypes. ICH-classified early BC patients usually receive adjuvant systemic treatment in addition to local treatment (surgery and radiation therapy) depending on their clinicopathological subtype. Current guidelines indicate that all patients with ER-positive disease should receive at least 5 years of adjuvant endocrine therapy (ET) [6–8]. One of the milestones of the current classification is the distinction between luminal A-like and luminal B-like tumors with significant different clinical outcomes, resulting in different indication of adjuvant cytotoxic therapy [8]. The indication for chemotherapy (CHT) in patients with luminal disease has traditionally been based on the prognostic factors of tumor size, histological grade, Ki-67 levels, ER and PgR expression, and number of involved lymph nodes. Nevertheless, studies suggest a high variability on criteria used to add CHT in the setting of luminal disease [9]. Previous studies showed that more than 60% of hormone receptor-positive BC patients receive adjuvant cytotoxic therapy. However, other authors provided evidence that only 4–5% of these women would likely benefit of this therapy [10]. Considering that serious and even life-threatening toxicities (bleeding, neutropenic fever, transfusion requirement, congestive heart failure, secondary malignancy, and peripheral neuropathy) occur in approximately 1–2% of patients, it is evident that conceiving a more specific prognostic system is mandatory in order to identify patients who can avoid CHT. The St. Gallen International Expert Consensus Panel adopted an intrinsic subtype-based approach for recommending adjuvant systemic therapies (i.e., ET, CHT, and anti-HER2 therapy) in early BC [8]. Although acknowledging the higher accuracy and reproducibility of gene expression assays, the panel recognized that they are not easily available for all BC patients, due to technical and especially economical reasons. As a result, immunohistochemistry (IHC)-based methods with antibodies recognizing ER and PgR, HER2, and Ki-67 are currently used as a surrogate for intrinsic subtypes, as detailed in Fig. 12.2.

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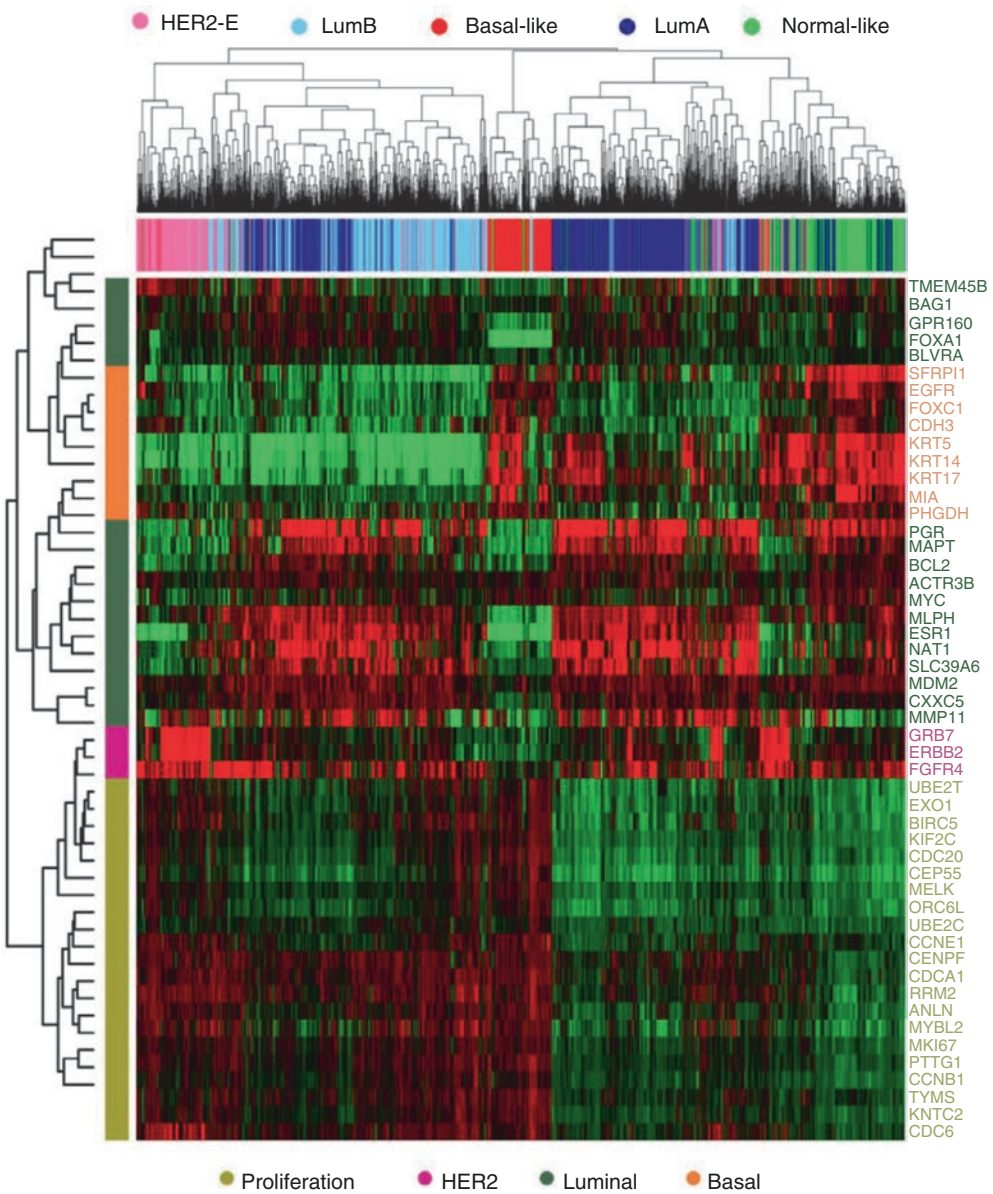

Fig. 12.1 Intrinsic subtype identification using the PAM50 classifier. The subtype calls of each sample are shown below the array tree. The expression values are shown as *red*/*green* according to their relative expression level [4]

Although the identification of intrinsic subtypes by IHC is widely distributed and relatively inexpensive, it is limited by interobserver variability and technical reproducibility [11]. Furthermore, several studies proved that IHC is not completely reproducible in identifying intrinsic subtypes, possibly due to the fact that four antigens do not fully recapitulate an intrinsic subtype originally identified by the expression of 50 genes: across the IHC-identified subtypes, the discordance rate is 38% for luminal A and 49% for luminal B [4]. Studies in the neoadjuvant setting provided indirect evidence that luminal A are less sensitive to CHT than luminal B tumors, thus achieving a significant lower rate of pathological complete response (pCR) when treated by different CHT schemes [4]. This is further sustained by the fact that pCR is prognostic in luminal B, but not in luminal A BC patients. The 2015 St. Gallen International Expert Consensus [12] recognized that luminal A-like BC are less responsive to CHT and should be therefore treated with ET only, with the exception of cases with extensive (four or more lymph nodes) axillary involvement. Oppositely, CHT in combination with ET is usually recommended for luminal B-like BC patients, unless they are bearing clinicopathological low-risk features, including T1 size, no or limited (1–3 nodes, pN1a) nodal involvement, absence of peritumoral vascular invasion, and very high ER/PgR and/or low Ki-67 values. Collectively, these data provided the rationale

for a better identification of which population of ER-positive (luminal) BC has a risk of relapse low enough to allow sparing a noneffective and potentially harming CHT treatment.

Intrinsic subtype	Clinico-pathologic surrogate definition
Luminal A	**'Luminal A-like'** *all of:* ER and PgR positive HER2 negative Ki-67 'low' Recurrence risk 'low' based on multi-gene-expression assay (if available)
Luminal B	**'Luminal B-like (HER2 negative)'** ER positive HER2 negative and *at least one of:* Ki-67 'high' PgR 'negative or low' Recurrence risk 'high' based on multi-gene-expression assay (if available) **'Luminal B-like (HER2 positive)'** ER positive HER2 over-expressed or amplified Any Ki-67 Any PgR
Erb-B2 overexpression	**'HER2 positive (non-luminal)'** HER2 over-expressed or amplified ER and PgR absent
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative

Fig. 12.2 Immunohistochemical surrogate definition of luminal intrinsic subtypes [8]

12.2 Multiparametric Molecular Markers: The Candidates

Using different techniques to measure mRNA levels, including RT-PCR and DNA microarrays, the assays shown in Fig. 12.3 have been basically designed to measure the risk of BC recurrence.

12.3 MammaPrint® 70-Gene Recurrence BC Assay

MammaPrint® is one of the first gene expression arrays approved by FDA for commercial use. It measures the mRNA expression of 70 genes, focusing primarily on proliferation, with additional genes associated with angiogenesis, metastasis, invasion, and stromal integrity [13–16]. This test was developed without an a priori knowledge of the role of the involved genes using a data-driven approach. Initially, about 5000 genes were found to be significantly deregulated across 78 BC patients treated at the Netherlands Cancer Institute [13]. All the patients were node negative, with tumor measuring less than 5 cm in diameter, were aged <55 years, and were selected irrespectively of their hormonal receptor status. Using a supervised classification method by correlating the expression of each gene with the disease outcome, the authors ended up with a core of 70 candidates bearing significant prognostic value [13]. The expression profile of these genes allowed to identify two patient subgroups, with "good prognosis" or "poor prognosis," having appreciable different risks to develop distant metastasis within 5 years. When the patients were classified according to the St. Gallen and National Institute of Health (NIH) criteria, the 70-genes score was found to be able to reduce the risk of overtreatment by

	21-gene RS (Oncotype Dx®)	Amsterdam 70-gene signature (Mammaprint®)	PAM50 (Prosigna™)	Rotterdam 76-gene signature	Genomic grade index	Breast cancer index	Endopredict®
Relevant EBC Population	ER+ HER2- Node-	Node- Tumor size ≤5 cm	ER+	Node-	ER+	ER+ Node-	ER+ HER2-
Tissue Required for Assay	FFPE	FFPE or frozen	FFPE	FFPE	FFPE or frozen	FFPE	FFPE
Assay Technique	qRT-PCR	Microarray	qRT-PCR	Microarray	Microarray	qRT-PCR	qRT-PCR
Demonstrated Analytic Validity	✓	✓	✓				✓
Demonstrated Clinical Validity	✓	✓	✓	✓	✓	✓	✓
Demonstrated Clinical Utility	✓		✓			✓	✓
Level of Evidence	IB	III	IB	III	III	IB	IB
Ongoing Studies	TAILORx, RxPONDER	MINDACT					

Fig. 12.3 Multigene prognostic tests for BC patients [Cobain EF, Hayes DF Curr Treat Options Oncol 2015]

25–30%. In particular, MammaPrint®, St. Gallen, and NIH systems assigned 40, 15, and 7% of the patients to the low-risk category, respectively. In a multivariable analysis, MammaPrint® showed a stronger independent prognostic ability than the matched clinicopathologic factors [17, 18]. The assay was then tested in a larger series of 295 patients including either ER-positive or ER-negative BC from the same institution, confirming its prognostic ability in predicting 10-year survival outcome. In particular, within the lymph node-negative sub-cohort (151 patients), the 10-year distant disease-free survival was 87% for the low-risk group and 44% for the high-risk group [19]. These data provided evidence that MammaPrint® was an high performing test in prognosticating ER-positive BC patients, outdoing current clinicopathological characteristics, while its clinical validity was much lower in the ER-negative setting, where nearly all of the patients were classified as high risk. Along this line, a number of retrospective analyses confirmed that only ER-positive patients within the high-risk category did benefit from adjuvant CHT [19–23] suggesting that MammaPrint® could also be used as a predictive tool. The first prospective study (RASTER), conducted in 16 community hospitals in the Netherlands, confirmed the feasibility of the 70-genes test [23] and evaluated its overall performance rate. The study enrolled 427 patients younger than 61 years with T1–T3, node-negative BC, irrespective to their hormonal receptor status. Patients received the adjuvant systemic treatment recommended by the 2004 Dutch Institute of Healthcare Improvement guidelines [24], also taking into account physicians' and patients' preferences. The results of the 70-genes classification were compared with Adjuvant! Online (AOL) [25]. This is a web-based tool for estimating risk of relapse and mortality and illustrating the benefits provided by various treatment regimens for newly diagnosed BC patients. The estimates for risk of death are derived from the Surveillance, Epidemiology, and End Results (SEER) data. AOL!® estimates recurrence by adding 14% to the mortality risk to account for the risk of contralateral breast cancer and local/regional events unlikely to result in breast cancer mortality. The estimates of treatment benefit are derived from available clinical trial results and data from the 1995 Overview meta-analyses of randomized adjuvant chemotherapy and hormone therapy trials for breast cancer [26], with supplemental information from the 2000 Overview [27]. AOL!® and MammaPrint® yielded to a 38% discordance rate in risk estimations, with most of the discordant cases being low risk according to the 70-gene signature and high risk according to AOL!®. The majority (98%) of these patients who did not receive adjuvant CHT showed an uneventful clinical course. Based on these data, it has been concluded that patients pertaining to the MammaPrint® low-risk group would spare CHT without any negative impact on recurrence rate [22]. The first independent validation study, performed by the TRANSBIG research consortium, used samples from 302 patients younger than 60 years and with node-negative, T1–T2 BC. This study confirmed the ability of MammaPrint® in discriminating patients' outcome with Hazard Ratio of 2.79 (95%CI, 1.60-4.87) and 2.32 (95%CI, 1.35-4.0) respectively, for distant metastasis and overall survival, outperforming clinicopathological characteristics [28]. According to the evidence that up to 25–30% of node-positive BC patients would remain free of distant metastasis even without any adjuvant CHT [29], a retrospective study selected 241 T1, T2, or operable T3, pN1a (1–3 metastatic lymph nodes) BC patients [20]. In this cohort, good-prognosis patients (41%) showed a 91% 10-year distant metastasis-free survival and a 96% BC-specific survival, respectively, while both the survival rates were 76% in the poor-prognosis group. Multivariable analysis showed that the 70-gene signature was the most powerful independent predictor for BC-specific survival, with a HR of 7.17, confirming its utility in identifying patients who can safely spare adjuvant CHT even if node positive [20]. Straver et al. [16] assessed the role of 70-gene assay in the neoadjuvant setting in a cohort of 171 patients with BC larger than 3 cm and/or with positive lymph nodes at diagnosis, finding that, as expected, 86% of the patients showed the high-risk signature. The rate of pCR was 20% for high-risk and 0% for low-risk patients [16]. In February 2007, the TRANSBIG consortium launched a multicenter, prospective, and randomized controlled study, the "microarray for node-negative disease may avoid CHT" trial, whose results have been eventually presented in 2016 at the AACR meeting and published soon thereafter [30, 31]. Out of the 11,288 patients enrolled, the trial assessed the risk in 6693 early BC patients by either AOL or the 70-gene assay. The 2142 (31.2%) patients with discordant results have been randomized to receive the adjuvant treatment dictated by AOL or gene expression profile, i.e., ET only for low-risk patients and ET + CHT for high-risk patients [30, 31]. The remaining patients, placed into the same risk category by both methods, were treated in accordance with the current guidelines (ET for the low-risk group and ET + CHT for the high-risk group). A total of 1550 patients (23.2%) were classified as high clinical risk and low genomic risk. At 5 years, the rate of survival without distant metastasis in this group was 94.7% (95% CI, 92.5–96.2) among those not receiving CHT. The absolute difference in survival rate between these patients and those who received CHT was 1.5% points [30, 31].

12.4 Oncotype DX® (Genomic Health 21-Gene Recurrence Score)

This assay evaluates the RNA expression of a panel of 21 genes (16 cancer-related genes and five reference genes) by RT-PCR, providing information about the 10-year risk of distant recurrence (DR). The 21-genes panel works in FFPE samples and includes genes involved in tumor cell proliferation (representing five of the 16 cancer-related genes),

invasion, HER2, and hormone response. The relative expression levels of these genes is calculated by a mathematical algorithm mostly weighting proliferation genes that generate the Recurrence Score (RS), expressed as a value between 0 and 100. RS provides a quantitative risk of distant recurrence and stratifies patients in three categories: low risk (RS < 18), intermediate risk (RS 18–30), and high risk (RS > 30) [32]. RS has been validated as an independent prognostic measure of the risk of recurrence for women with ER-positive, lymph node-negative early BC treated by ET only, outperforming traditional clinicopathological characteristics of patient age, tumor size, and grade [32, 33]. In 2010, a retrospective study examined specimen collected within the ATAC trial with the objective to evaluate the prognostic value of the Oncotype in the postmenopausal setting [34]. The ATAC trial evaluated the efficacy and safety of 5 years of anastrozole, tamoxifen, or the combination of both in postmenopausal women and selected more than 5000 women with localized invasive BC and ER-positive disease [35]. 76% of the specimens from this collection was then used to confirm the performance of RS in elderly patients, demonstrating that RS was an independent predictor of recurrence in both nodes-negative and nodes-positive patients [34]. Moreover, the RS was found to be a strong predictive factor of benefit from cyclophosphamide, doxorubicin, and fluorouracil and fluorouracil (CAF) in ER-positive, node-positive, postmenopausal BC patients. Patients classified as low risk did not derive any benefit from CHT, while a significant advantage from treatment with CAF was observed in patients with a high RS [36]. Likewise, in a cohort of 89 patients with locally advanced BC treated preoperatively with paclitaxel and doxorubicin, the probability of pCR was shown to increase along with RS [37]. Oncotype DX® RS is widely used in the USA, allowing to spare CHT in approximately one third of the cases [38–40] and resulting in overall cost reduction for the health system [41]. A prospective clinical study, the Trial Assigning IndividuaLized Options for Treatment (Rx) (TAILORx), is currently seeking to incorporate the Oncotype DX® test into clinical decision-making, in order to spare women unnecessary treatment if CHT is not likely to be of substantial benefit [42]. TAILORx recruited more than 10,000 women with node-negative, ER- and/or PgR-positive, HER2-negative invasive BC. For this trial, the original RS threshold values have been modified as follows: below 11 (vs. <18) for the low-risk group, from 11 to 25 (vs. 18–30) for the intermediate-risk group, and above 25 (vs. ≥31) for the high-risk group. Low- and high-risk patients have been assigned to ET only or ET + CHT, respectively, while patients with an intermediate (11–25) RS have been randomly assigned to receive ET + CHT or ET only. Sparano et al. [43] recently reported an interim analysis of the TAILORx trial. Patients had tumors measuring 1.1–5.0 cm in the greatest dimension (or 0.6–1.0 cm and G2/G3 tumor grade) and met established guidelines for the consideration of adjuvant CHT on the basis of clinicopathological features. A total of 1626 patients were assigned to receive ET without CHT if they had a recurrence score of 0–10, indicating a very low risk of recurrence. The 5-year rate of invasive DFS was 93.8% (95% CI 92.4–94.9), the rate of freedom from recurrence of BC at a distant site was 99.3% (95% CI, 98.7–99.6), and the rate of OS was 98.0% (95% CI, 97.1–98.6). These data supported the application of 21-genes assay to select patients who may be safely spared CHT treatment [43]. Several studies argued that RS does not provide prognostic information beyond traditional clinicopathological characteristics (i.e., ER/PR receptor, Ki-67 labeling index, and tumor grade), criticizing the cost-effectiveness of the Oncotype DX test [44–52]. In particular, Gage et al. [53] recently interrogated a population of 540 BC patients, reporting that 55% of the study population that would have met the criteria for Oncotype DX® testing (node-negative, ER-positive, HER2-negative invasive BC) would be easily classified in the low- and high-risk category by traditional tools, thus not needing additional information. Specifically, patients with high tumor grade or low (<20%) ER immunoreactivity should be considered at high risk of distant relapse, while patients with low tumor grade and highly ER and PgR express tumors at low risk. The authors concluded that only patients bearing intermediate features would benefit of the Oncotype DX® testing, leading to significant cost savings [53]. Taking into account that RS increases in relation to the levels of proliferation genes and that patients with higher RS benefit from adjuvant CHT, Baxter et al. [54] investigated the prognostic relevance of traditional biomarkers of proliferation (mitotic count and Ki-67 labeling index) in 226 ER-positive, HER2-negative, T1/T2, node-negative BC patients referred to British Columbia Cancer Agency in 2007–2011. The authors found that tumors with a low/intermediate Nottingham grading or low mitotic count were unlikely to be classified as high risk by Oncotype DX®, suggesting to test only patients with high histological score [54].

12.5 EndoPredict®

The EndoPredict® (EP) assay was developed for early, ER-positive, HER2-negative BCs, in order to identify patients with a low rate of recurrence without adjuvant cytotoxic therapy. Based on a RT-PCR method, EP evaluates the expression levels of eight cancer genes (AZGP1, BIRC5, DHCR7, IL6ST, MGP, RBBP8, STC2, UBE2C) and three control genes (CALM2, OAZ1, RPL37A) in FFPE tissue. These genes are related to tumor proliferation and to hormone receptor activity, but do not include ESR1, PgR, or HER2, at variance with Oncotype DX® and PAM50 assays. EP classifies patients treated with adjuvant ET only into a low- or a high-risk category, and it is feasible in a decentralized setting [55–57]. The EP score ranges between 0 and 15 with a threshold of 5 to discriminate between the low- and

high-risk categories. EP was developed and validated in over 1000 postmenopausal, node-negative and node-positive ER-positive and HER2-negative BC samples, retrospectively collected in prospective clinical trials [58]. Continuous EP score proved its prognostic role for distant recurrence, outperforming the established clinicopathological variables of ER immunoreactivity, Ki-67 labeling index, and AOL. The EP score has been subsequently integrated by the clinical characteristics of nodal status and tumor size to create a linear model risk score called EPclin that in turn proved to be a powerful prognostic marker, resulting in a 10-year recurrence rate of 4% for the EPclin low-risk group and 22–28% for the high-risk group [59]. EPclin identifies a subgroup of patients with an excellent long-term prognosis after 5 years of ET, confirming its prognostic ability for both early and late relapse and suggesting that the low-risk patient subgroup might not need an extended ET [58]. The EPclin score has also been found to outperform purely clinical risk classifications (St. Gallen, German S3, and NCCN). Among 1702 ER-positive/HER2-negative, postmenopausal women treated with exclusive ET, 58–61% of patients classified as high/intermediate risk according to clinical guidelines were reassigned to the low-risk group by the EPclin score [60]. In a retrospective study dealing with 167 patients with ER-positive, HER2-negative BC, EPclin score led to a change of the planned therapy in 37.7% of patients, shifting to CHT in 12.3% and to exclusive ET in 25% [61]. The interaction between EP score and CHT has also been investigated: Bertucci et al. [62] collected 553 ER-positive and HER2-negative BC pretreatment core biopsies samples for which documentation of pathological response to anthracycline-based neoadjuvant CHT was available, finding that the high-risk group had a higher pCR rate than the low-risk group (17 vs. 7%). Martin et al. [63] reported a prospective-retrospective clinical validation trial designed to investigate whether EP can safely be used to identify node-positive BC patients who can avoid CHT. In this cohort of 1246 ER-positive, HER2-negative, node-positive, CHT (5-fluorouracil, epirubicin, and cyclophosphamide with or without 8 weekly courses of paclitaxel)-treated BC patients, 25% were classified as low risk on the basis of the EP score. In this subgroup, 93% of the patients showed a distant metastasis-free survival, compared to 70% in the high-risk group.

12.6 PAM50® and Risk of Recurrence (ROR) Score

This assay was developed to classify tumors according to the intrinsic subtype (see above) and to improve the classification concordance reported by investigators and is based on the relative expression of 50 genes [3]. Provided that the data are normalized, the test is considered a robust assay with a high concordance between laboratories [64]. The PAM50 classifier was validated in a cohort of 348 patients receiving tamoxifen, where it was found to outperform IHC in providing prognostic information and in predicting tamoxifen efficacy [65]. In a population of 151 ER early-stage BC patients, PAM50 achieved a good level of agreement with Oncotype DX® in identifying both high (luminal B and RS > 31)- and low-risk groups (luminal A and RS < 18) [66]. Within the group of Oncotype DX® intermediate RS, PAM50 classified 59% of the patients as luminal A, 33% as luminal B, and 8% as HER2 enriched. Moreover, Ki-67 labeling index was found to be reliable in distinguishing luminal A from luminal B and low-risk from high-risk RS tumors but not between the intermediate- and low-risk RS categories [66]. Adopting an algorithm that incorporates gene expression data, intrinsic subtype, and tumor size, Parker et al. [3] created the risk of recurrence (ROR) score (Prosigna), which stratifies patients in high, medium, and low subsets. The clinical utility of PAM50 and ROR score as a prognostic tool has been repeatedly reported in the ER-positive, HER2-negative setting [65, 67, 68].

12.7 Rotterdam 76-Gene Signature

This assay was developed at the Erasmus University Cancer Center in Rotterdam and made commercially available in 2005. The 76 genes included in this assay are mainly related to proliferation. The test was developed from the analysis of 115 women with node-negative BC (ER positive and ER negative), not receiving any adjuvant treatment and followed for more than 8 years, and differentiates patients in two categories, i.e., good signature or poor signature [69]. It has been reported to be highly predictive of distant relapse at 5 and 10 years. Desmedt et al. [70] found in a cohort of 198 node-negative untreated patients that the 5- and 10-year time to distant metastasis was 98 and 94% for the good profile group and 76 and 73% for the poor signature group, respectively. These data stemmed from retrospective analyses and still need to be confirmed in prospective randomized studies. The analytic validity, clinical utility, and reproducibility across different laboratories have not yet been confirmed.

12.8 Genomic Grade Index

Histologic grade is one of the best-established prognostic biomarkers in BC, providing reliable information regarding tumor behavior [71, 72]. However, the Elston-Ellis histological grading system shows low reproducibility among pathologists [73] and does not provide clear prognostic information for patient with grade 2, which represents the majority of cases [74]. The genomic grade index (GGI) was developed

with the aim of grading breast tumors more accurately than the conventional histological grade in the ER-positive, HER2-negative setting [75]. It was developed in 189 BC patients and validated in an independent cohort of 597 cases. The authors created a two-tier classification system based on the differential expression of 97 genes mainly involved in cell cycle regulation and proliferation. The level of expression of these genes was found to reclassify grade 2 tumors into high and low genomic grade category. High GGI score patients were associated with a higher risk of recurrence than low GGI score patients (HR = 3.61, 95% CI = 2.25–5.78) [76]. Loi et al. [77] demonstrated the prognostic ability of GGI in stratifying luminal tumors, reporting that luminal A and B tumors fall into the GGI low risk and high risk, respectively. The role of GGI in predicting pathological response to neoadjuvant CHT was investigated in 229 fine-needle biopsies of BC patients treated with a taxane- and anthracycline-containing neoadjuvant therapy. In this study, a high GGI score was an independent predictor of response to CHT [78].

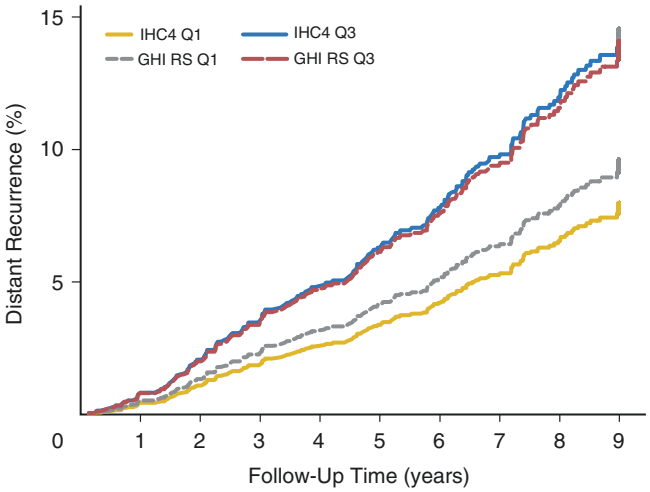

Fig. 12.4 Example of predicted time to distant recurrence for a node-negative postmenopausal patient with a G3 1–2 cm tumor treated with anastrozole who is at either the 25th (quartile 1 [Q1]) or 75th (Q3) percentile of the IHC4 score (score for four immunohistochemical markers: estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, and Ki-67) or the Oncotype DX® Genomic Health recurrence score (GHI-RS) [82]

12.9 BC Index (BCI)

It is a RT-PCR-based assay working in FFPE samples, based on the HOXB13-to-IL17BR expression ratio (H:I ratio) and the molecular grade index (a five-gene molecular grade index, primarily consisting of proliferation-related genes) [79]. This assay was developed using a cohort of ER-positive tamoxifen-treated BC patients and has been shown to provide an individual risk of distant BC recurrence based on a continuous risk model [80]. The main strength of the BCI is its capability of predicting the risk of both early (within 5 years) and late (10 years) recurrences in ER-positive, node-negative BC. Indeed, the assay was retrospectively evaluated in two cohorts of 317 and 358, ER-positive, node-negative tamoxifen-treated patients. In both cohorts, continuous BCI was found the most significant prognostic factor beyond standard clinicopathologic factors, both for early and late events [81].

12.10 Comparative Evaluation of Prognostic Performance of Multigene Tests and Clinicopathological Characteristics

Most of the comparative data on multigene prognosticators have been obtained by Dr. Dowsett lab taking advantage of the samples prospectively collected within the TransATAC, the translational substudy of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial [35]. The ATAC trial randomized ER-positive, HER2-negative BC patients to receive exclusively tamoxifen or anastrozole for 5 years, with distant relapse as the primary end point. Cuzick et al. [82] comparatively analyzed the performance of Oncotype DX RS and IHC4 score (resulting from the immunohistochemical evaluation of ER, PgR, HER2, and Ki-67 integrated into the IHC4 score). The authors found that the information provided by the IHC4 score and Oncotype DX® were similar and that little additional prognostic value was seen combining both scores (Fig. 12.4).

Dowsett et al. [68] then compared the prognostic relevance of PAM50 ROR score with Oncotype DX RS and IHC4 in 940 ER-positive BC patients. The ROR score added significant prognostic information for distant relapse in the whole population ($p < 0.001$) and in HER2-negative/node-negative patients. Likewise, PAM50 ROR provided additional prognostic information beyond Oncotype DX® RS in the overall population and within each subgroup. Interestingly, relatively similar information was provided by ROR and IHC4 in all patients. Buus et al. [83] recently compared the prognostic information provided by Oncotype DX®, EP/EPclin, and clinical treatment score (CTS, obtained by integrating the prognostic information from nodal status, tumor size, histopathological grade, age, and anastrozole or tamoxifen treatment) in 928 ER-positive, HER2-negative BC patients enrolled in the anastrozole and tamoxifen arms of the ATAC trial, with distant relapse-free survival as the primary end point. In the overall population, EP and EPclin provided substantially more prognostic information than Oncotype DX RS, especially with regard to the risk of late relapse and in node-positive patients. In a prospective comparison study, conducted on 665 patients with ER-positive, node-negative BC patients, BCI was com-

pared with the Oncotype DX® 21-genes recurrence score. Both the assays demonstrated significant prognostic ability for early distant recurrence, while only BCI was significant for late distant recurrence [84].

12.11 Concordance Among Gene Expression Tests

It has been demonstrated that gene expression-based tests show only moderate concordance. For example, Oncotype Dx® and EndoPredict® had a 24% discordance in a study reported by Dowsett et al. [83]. This level of concordance is similar to that reported between Oncotype Dx- and Ki67-based risk algorithms. The fact that even gene expression-based tests show a nonnegligible discordance rate when ran in a single patient, coupled with the reality of medical practice in countries where gene expression-based tests are not affordable owing to high cost (in Italy, the out-of-pocket cost for patients is >$4000), underlines the importance to limit the use of gene expression tests to subgroups of ER-positive BC patients and to put efforts to validate Ki-67 (as a surrogate of proliferation) as a predictive marker for CHT benefit.

12.12 Toward a Rationale Use of the Multigene BC Prognosticators

The aforementioned gene expression-based prognostic tests are highly reliable in informing on ER-positive/node-negative BC patients prognosis, and most of them achieved a level of evidence 1B, being validated in samples retrospectively collected within prospective randomized clinical trial. Furthermore, MammaPrint® and Oncotype DX® have been recently validated in prospective clinical trials using molecular data as a randomization factor. This notwithstanding, the multigene tests are not widely used in the daily practice, especially for their high costs and the need of sample centralization. A meta-analysis of BC microarray gene expression profiling data [85] demonstrated that they are all basically looking at the same process: tumor cell proliferation. In other words, they essentially discriminate ER-positive BCs with low proliferation (luminal A intrinsic subtype) and low clinical risk, from ER-positive BCs with high proliferation (luminal B intrinsic subtype) and high clinical risk [85–87]. Multigene tests are used for assessing whether a patient with an early ER-positive BC should receive CHT, and in this regard they may be considered as a potential biomarker. Although their analytical and clinical validity has been convincingly demonstrated, uncertainties remain concerning their clinical utility. A biomarker has clinical utility if its application is associated with a significant survival benefit: it should outperform preexisting clinicopathological indicators or, alternatively, provide comparable information at lower cost, less invasively, or with less morbidity. Nevertheless, these advantages are still not enough for achieving clinical validity: as stated by the recently issued ASCO guidelines for the use of biomarkers in early BC [88], "the magnitude of the benefit must be clinically meaningful and outweigh risks, costs, and/or inconvenience associated with use of the test and the degree of benefit required to recommend for or against a treatment must be tempered with clinical judgment and patient perspective." For example, giving adjuvant CHT to triple negative, node-positive BC patients is of clinical utility beyond any doubt: the 10-year likelihood of incurable distant recurrence in this setting would exceed 50% in the absence of adjuvant CHT that indeed reduces the risk of recurrence by 30%, with a 15–20% absolute benefit and an odd of fatal, life-threatening, or permanent life-changing toxicities accounting for 2–3%. On the other hand, the 10-year risk of recurrence for luminal A-like BC patients (ER/PgR highly expressed, HER2 negative, <20% Ki-67, and/or G1) does not exceed 10% with ET only. This means that adding CHT, that would reduce the risk of recurrence by 30% in this setting as well, would yield to a 3% absolute benefit, which is roughly the same figure of patients potentially harmed. The 2015 St. Gallen International Expert Consensus [12] recognizes that luminal A-like BCs are less responsive to CHT and should be therefore treated with ET only, with the exception of cases with extensive (four or more lymph nodes) axillary involvement. Oppositely, CHT in combination with ET is usually recommended for luminal B-like BC patients, unless they are bearing clinicopathological low-risk features, including T1 size, no or limited (1–3 nodes, pN1a) nodal involvement, absence of peritumoral vascular invasion, and very high ER/PgR and/or low Ki-67 values, as well as multiparameter molecular markers of favorable prognosis [12]. The fact that these multigene tests have been validated only for node-negative patients (with the exception of MammaPrint® that has been used in 1–3 node-positive patients within the MINDACT trial) is anything but trivial in the clinical practice. ET is usually delivered to patients with luminal tumors characterized by favorable prognostic markers, including high levels of ER/PgR immunoreactivity, small T size (T1/T2), and absence of lymph node involvement, thus questioning the usefulness of running multigene tests in N0 luminal patients. Oppositely, patients with extensive lymph node involvement usually receive CHT irrespective of the biology of their tumor. As a consequence, multigene tests would be clinically useful specifically in patients with 1–3 positive lymph nodes. In this regard, Gnant et al. [89] recently investigated the prognostic role of PAM50 ROR in 543 patients with one to three node-positive BC treated with 5 years of adjuvant ET only within two phase III adjuvant trials: ABCSG-8 and ATAC. The authors found that the patients with one positive

lymph node classified as low risk by PAM50 ROR had a 6.6% risk of distant recurrence at 10 years (95% CI 3.3–12.8%). By contrast, low-risk patients with two or three positive lymph nodes nearly doubled the risk of distant recurrence to 12% (95% CI 6.6–22.8%). Assuming that CHT could reduce the risk of recurrence by 30%, these data prompt to speculate that ROR low-risk patients should receive CHT when 2–3 lymph nodes are involved, while the benefit of any further treatment in patients with just one metastatic lymph node would be negligible. Interestingly, patients without any lymph node involvement are more frequently classified as low risk by different multigene tests. Notwithstanding these data, the putative value of genomic tests in decision-making of luminal BC patients with 1–3 node-positive disease remains to be established. The MINDACT trial [30] reported that the 5-year rate of survival without distant metastasis in the clinical high-risk/molecular low-risk group was 94.7%, indipendently of the occurrence of lymph node metastasis. There are three further prospective phase III randomized trials (TAILORx and RxPONDER using Oncotype DX® and ASTER 70s using genomic grade) currently addressing the role of multigene tests in predicting adjuvant CHT benefit also in patients with up to three node-positive luminal BC, providing soon level 1 evidence on their clinical utility in daily practice. For the time being, ASCO guidelines recommend not to use multigene tests for ER-positive BC patients with lymph node involvement [88].

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Special Types of Breast Cancer and Non-epithelial Tumors

13

Giovanni Mazzarol and Sara Pirola

13.1 Introduction and Historical Perspective

Since the early twenties of the past century, when according to Dr. Borst only five breast tumor subtypes had been identified, the histopathological classification of breast carcinomas has been profoundly modified, in order to refine its diagnostic efficiency and to embody the results flourishing from basic science [1, 48]. Histopathology has been therefore relentlessly evolving for the accomplishment of two main tasks: providing prognostic information and predicting the response to surgical and medical treatments. In the early seventies, Dr. Haagensen pointed out in his book entitled *Diseases of the Breast* his "…hope to sort out from among them (breast tumors)…, additional characteristic types of breast carcinomas," in an attempt to ascertain their clinical-pathological correlation. He emphasized the need to consider in situ lesions as "fully malignant," recommending the same "drastic" surgical cure usually applied to invasive tumors [2]. This attitude clearly illustrates how physicians have been tailoring the treatment of breast cancer to histopathological features since the beginning of modern oncology.

Pathologists have been deeply involved into breast cancer care by then, developing the concept of invasive breast cancer of special type, which carries obvious "useful clinical correlates and prognostic implications" [3]. Along this line, Japanese authors developed a morphological classification dissecting tubule-papillary, solid-tubular, and scirrhous patterns according to their private risk of relapse [4]. The painstaking evaluation of tumor histology allowed the recognition of different coexisting patterns [5]. In particular, combined features of special type carcinomas have been described in up to 30% of breast carcinomas of NST (no special type). The frequent occurrence of morphological tumor heterogeneity prompted pathologists to recognize mixed types of breast tumors, which may hinder the clinical relevance of histological classification. The overall percentage of the special component has been described according to different series and authors, ranging from over 50% to at least 90% [3]. Actually, the lack of agreement in the cutoff by which a specific histological subtype should be considered as predominant has weakened the clinical impact of subgrouping breast carcinomas [6, 7].

13.2 Mucinous Carcinoma

Mucinous carcinomas have pushing margins with typical gelatinous, soft cut surface.

The neoplastic cells have intracellular mucin with solid, micropapillary, cribriform, and tubular formations, floating in pools of extracellular mucin. "Signet ring" cells may also be present, rarely being the predominant feature [46]. Multiple sections are required in paucicellular form to detect the neoplastic cells to establish the diagnosis. Delicate bands of fibrovascular connective tissue can be observed within the mucous lakes. This characteristic histology should be present in at least 90% of the tumor. Almost all tumors express strong ER and PgR and rarely overexpress HER-2/neu oncoprotein. According to different series, the frequency is up to 2%. They have excellent prognosis with a 10-year overall survival up to 80–100% [8, 9] (Fig. 13.1a).

13.3 Tubular Carcinomas

They are ill-defined, usually small neoplasm with stellate appearance. They are characterized by an irregular haphazard collection of angulated, oval, or elongated well-formed tubules, with a central lumen; typically, they have a single

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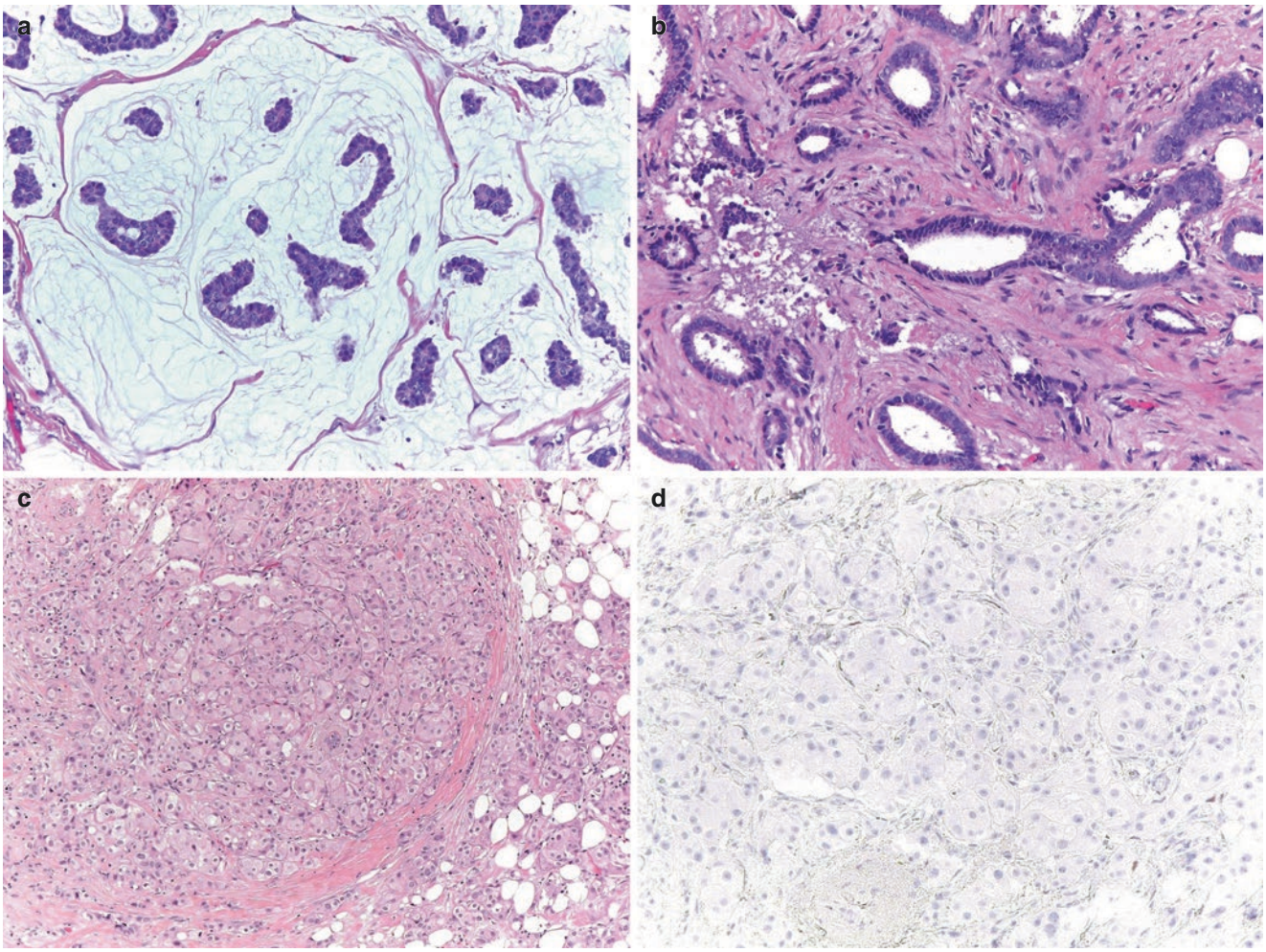

Fig. 13.1 (**a**) Mucinous carcinoma shows neoplastic cells floating in mucous lake. (**b**) Tubular carcinoma with angulated single layer glands in desmoplastic stroma. (**c**) Apocrine carcinoma with eosinophilic large cytoplasm. (**d**) Negative estrogen receptor immunohistology in the very same apocrine carcinoma depicted in (**c**)

layer of small, monomorphic epithelial cells often showing apical "snouts." They show desmoplastic changes and/or stromal elastosis. Tubular carcinoma shows low cytologic atypia with rare mitoses. Calcifications are often present. These features should be present in more than 90% of the lesion. Conventional types of ductal intraepithelial neoplasia (DIN) may be present, and coexistent columnar cell lesions including flat epithelial atypia and lobular neoplasia are common in the proximity of tubular carcinoma. They extensively express ER and PgR and they do not overexpress HER-2/neu oncoprotein. They account of less than 2% of carcinomas, and the survival at 10 years is up to 99–100% [6, 10] (Fig. 13.1b).

13.4 Apocrine Carcinoma

They may show a brownish-tan cut surface. They have cytological features of apocrine cells, and two types of cells could be observed: type A cells (large, with abundant eosinophilic granular cytoplasm with enlarged rounded hyperchromatic nuclei and prominent nucleoli) or type B cells with foamy cytoplasm containing lipid droplets resembling histiocytes or sebaceous differentiation. Tumor cells with bizarre, multilobulated nuclei may be present. The apocrine morphology needs to be seen in more than 90% of the cancer cells. An intraepithelial apocrine component (DIN) with high nuclear grade is often present. They typically express androgen recep-

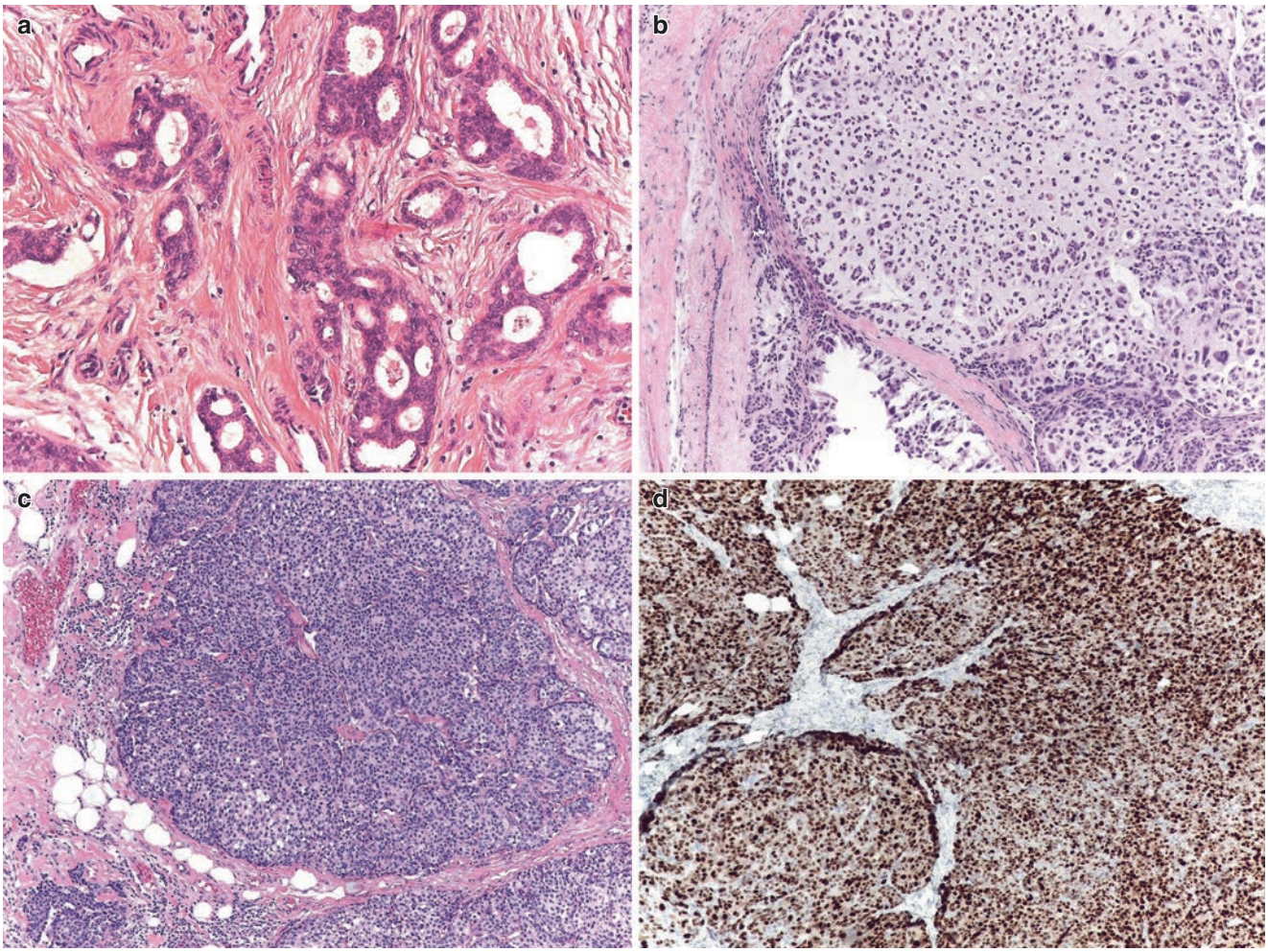

Fig. 13.2 (**a**) Cribriform carcinoma with typical fenestrated glands. (**b**) Metaplastic carcinoma with direct transition from epithelial cells to mesenchymal component (matrix-producing). (**c**) Papillary carcinoma showing solid pattern with fibrovascular core. (**d**) Positive estrogen receptor immunohistology in the very same papillary carcinoma depicted in (**c**)

tors and are triple negative, even though sometimes they may overexpress HER-2/neu oncoprotein. According to different series, they account to 1–4% of the carcinomas. The pure form has a 10-year survival >95% [11, 12] (Fig. 13.1c, d).

13.5 Cribriform Carcinoma

They show angulated and fenestrated cribriform glands as in ductal intraepithelial neoplasia (DIN) of cribriform type with low or intermediate nuclear grade cellularity. Osteoclastic-like giant cells have been reported in some cases. Mitosis is rare. In the mixed cribriform carcinoma form, more than 50% show a cribriform pattern, but areas (10–49%) of non-tubular less differentiated type are also present. In the vast majority of cases, adjacent DIN with cribriform and micropapillary growth pattern is present. They express ER and PgR but usually do not overexpress HER-2/neu oncoprotein. In the pure form, they represent up to 4% of breast carcinomas, with a 10-year survival of 90–100% [6, 13] (Fig. 13.2a).

13.6 Medullary Carcinomas

They are well circumscribed, with a soft, gray/tan cut surface. Medullary carcinomas have histologic circumscription with pushing, expansive margins (smooth, rounded contour). They grow in a syncytial pattern of solid clusters of tumor cells forming anastomosing cords and sheets. Neoplastic cells have severe nuclear atypia, prominent nucleoli, and

indistinct cell borders with high mitotic count. The lymphoplasmacytic infiltration (composed almost entirely of either lymphocytes or plasma cells) is often present throughout the tumor. They are triple-negative carcinomas with a good prognosis if strict histologic criteria are accomplished (up to 95% survival) [14, 15].

13.7 Metaplastic Carcinomas

They are carcinomas with a variable percentage of mesenchymal differentiation. They could be subdivided in two categories: carcinomas with squamous and/or spindle cell metaplasia (low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, and spindle cell carcinoma) and carcinomas with heterologous metaplasia.

Carcinomas with heterologous metaplasia are poorly differentiated duct carcinomas associated with mesenchymal elements, most commonly chondroid, osseous, and rhabdomyoid, but also lipomatous and angiomatous. The heterologous elements can be either well differentiated with minimal atypia or sarcomatous as for tumors originating in soft tissues. If there is a direct transition from epithelial to the mesenchymal components without an intervening spindle cell component, the term "matrix-producing carcinoma" has been used (Fig. 13.2b).

Metaplastic carcinomas are triple negative, although occasional tumors with focal positivity for ER and/or HER-2/neu are encountered in the mesenchymal component. The frequency is up to 5% of the carcinomas, and the overall survival at 5 years is 18–81% according to stage at the presentation [16, 17].

13.8 Squamous Cell Carcinomas

Squamous cell carcinomas arising in the breast parenchyma are exceedingly rare and usually represent metastatic squamous carcinoma. In their pure form, they often appear as cystic lesion with keratin debris, simulating necrosis. Intercellular bridges and keratin pearls are observed and spindle or acantholytic in appearance as well. They are triple-negative carcinomas with a survival at 5 years ranging from 81 to 46.9% if metastases (both regional and distant) are absent or present at the time of diagnosis [18].

13.9 Papillary Carcinoma

Papillary carcinomas have a predominantly papillary morphology (>90%) harboring papillae formed by malignant epithelial cells and fibrovascular cores. If the individual papillary fronds become crowded and are not separated by spaces, the term solid papillary carcinoma is used (Fig. 13.2c, d). An intraepithelial component (DIN), often also demonstrating a papillary architecture, is usually present. Neuroendocrine features have been consistently reported. ER and PgR positivity and HER-2/neu negativity is the most frequent phenotype. The prevalence varies according to different series from 0.04 to 2.7% with an overall survival of >90% at 5 years [19].

13.10 Micropapillary Carcinoma

Micropapillary carcinomas are arranged in micropapillary, tubuloalveolar, or morular clusters and lie within optical clear stromal spaces (shrinkage artifact), simulating lymphatic/vascular spaces. They lack fibrovascular cores, sometimes containing mucinous material. Typically, the cells have the apical surface polarized to the outside, finely granular or dense eosinophilic cytoplasm, and intermediate-to-high-grade nuclei with frequent mitoses. The majority of tumors are associated with an intraductal component of micropapillary and cribriform patterns and show extensive peritumoral vascular invasion paralleled by a prevalence of axillary lymph node metastasis significantly higher than ductal carcinoma NST. They variably express ER, PgR, and HER-2/neu [20].

13.11 Salivary Gland-Type Carcinomas

The most common subtype of salivary gland-type carcinomas is represented by adenoid cystic carcinoma. These tumors are usually well circumscribed with small cystic areas. They are formed by adenoid and basaloid cells forming true glandular spaces and pseudo-lumina. Sebaceous cells and squamous metaplasia of luminal cells may be present. They grow with an irregular, infiltrating pattern showing solid, cribriform, and trabecular-tubular arrangements, even if a mixture pattern may often be observed. As for their salivary gland counterpart, high-grade carcinomas have >30% of solid growth, while well-differentiated forms show exclusively a cyst and glandular appearance. Tumors with an intermediate differentiation show <30% of solid pattern. Mitotic count is usually low [21]. They are triple-negative carcinomas with an excellent prognosis when well differentiated (up to 90%) at 10 years. The remaining subtypes of salivary gland-type carcinomas, i.e., mucoepidermoid and acinic cell carcinomas, are exceedingly rare and resemble morphologically their salivary gland counterpart.

13.12 Rare Types of Breast Carcinoma

Few cases of tubulo-lobular carcinoma have been reported. These tumors are classic type of lobular carcinomas intermingled with well-formed single layer glands. Other very uncommon breast cancer subtypes are represented by lipid-

rich carcinoma, which shows extensive foamy appearance due to intracytoplasmic lipid accumulation, glycogen-rich carcinoma, secretory and hypersecretory carcinoma, and osteoclast-like giant cell carcinoma. Neuroendocrine small cell carcinomas have morphological features similar to their lung counterpart.

13.13 Non-epithelial Tumors

Primary mesenchymal tumor of the breast represents a heterogeneous group of neoplasm by far less frequent than pure epithelial neoplasm. Non-epithelial neoplasm could be subdivided in mixed (fibroepithelial neoplasms) and in pure mesenchymal form [22]. The most relevant entities of the first group are fibroadenomas and phyllode tumors. Fibroadenoma is a well-circumscribed biphasic (fibroepithelial) neoplasm showing stromal proliferation around glands (pericanalicular pattern) or compressing cleft-like ducts (intracanalicular pattern). The ducts are lined by two cell layers of luminal epithelial cells and myoepithelial cells. Fibroadenomas and phyllode tumors could be considered a continuum degree of progressive malignancy of the stromal component, which in the high-grade phyllode tumors is definitely sarcomatous (so-called cystosarcoma phyllodes). The stroma is loosely cellular, with regular spindled cells and collagen, and it may sometimes exhibit multinucleated giant cells, extensive myxoid changes, or hyalinization [23].

Areas of stromal hypercellularity may be seen within a fibroadenoma, leading to a diagnosis of cellular fibroadenomas because the typical leaflike architecture of phyllode tumors is absent or focal. Mitotic figures are uncommon. The epithelial component of fibroadenoma can show varying degrees of epithelial hyperplasia, particularly in young women. Squamous or apocrine metaplasia may also occasionally be observed. Whenever papillary apocrine changes, cysts, epithelial calcifications, and sclerosing adenosis occur, these tumors have been classified as complex fibroadenomas. Rarely, atypical ductal hyperplasia, lobular neoplasia, ductal intraepithelial neoplasia (DIN), or carcinoma may occur within fibroadenomas [24].

Benign phyllode tumors resemble intracanalicular fibroadenomas, and the hallmark of the tumor is the formation of stromal leaflike processes protruding into cystic spaces. Phyllode tumors are classified as benign, borderline, and malignant. In benign tumors, the mitotic count should not exceed 2 × 10 HPF. Borderline and malignant phyllode tumors are distinguished on the basis of the degree of stromal cellularity, stromal atypia, stromal overgrowth, tumor borders, and mitotic activity (3–9 × 10 HPF in borderline and >9 in malignant phyllode tumors). Local recurrences can occur in all types of phyllode tumors, with the highest prevalence for the malignant type, and distant metastases have been reported almost exclusively in malignant tumors [25, 26].

The second group of pure mesenchymal tumors mirrors the morphological features of their counterparts primarily arising in soft tissues [27]. Breast sarcomas must be differentiated from metaplastic carcinoma, due to their different surgical and clinical management. As a matter of fact, the sarcomatous component of a triple-negative carcinoma with extensive metaplastic features may outgrow the epithelial component, thus leading to a misdiagnosis of primary pure sarcoma. Therefore, focal remnants of carcinoma should be scrutinized in tumors showing prominent mesenchymal differentiation.

Vascular lesions include benign hemangioma and angiomatosis, atypical vascular proliferations, and angiosarcomas. Angiosarcomas may develop following radiation therapy for breast cancer or, less commonly, as primary neoplasms arising in patients with no prior history of radiation [28–31].

Tumors showing adipocyte differentiation include lipoma, a benign tumor composed of mature adipocytes without atypia, sometimes incorporating small vessels (angiolipoma), and liposarcoma that represents its malignant counterpart [32].

Schwannoma and neurofibroma of the breast derive from the sheath of peripheral nerves; most of them arise in the mammary subcutaneous tissue, even if parenchymal lesions have also been described [33].

Primary granular cell tumor of the breast is a benign neoplasm derived from Schwann cells of peripheral nerves and composed of compact nests of cells with prominent eosinophilic cytoplasmic granules, which are PAS positive and strongly immunoreactive for CD68 and S100 protein [34].

Myofibroblastoma is a benign, well circumscribed, pseudoencapsulated mammary stromal spindle cell tumor with prominent myofibroblastic differentiation, immunoreactive for desmin, smooth muscle actin, and CD34. They show broad bands of hyalinized collagen in the absence of any mammary duct and lobules [35].

Desmoid-type fibromatosis of the breast is a locally infiltrative, histologically low-grade proliferation of spindle cells and collagen. It rarely occurs within the breast parenchyma, frequently arising from the pectoral fascia [36].

Nodular fasciitis is a self-limiting, mass-forming fibroblastic/myofibroblastic proliferation. Inflammatory myofibroblastic tumor is a usually low-grade neoplasm composed of myofibroblastic spindle cells with prominent admixed inflammatory cells, most commonly plasma cells [37].

Pseudoangiomatous stromal hyperplasia is a benign disease in which the stromal cells form a complex pattern of anastomosing empty spaces in a dense collagenous stroma coexisting with duct and lobular epithelium. The spaces rarely contain a few red blood cells. Myofibroblasts (usually CD34 and calponin immunoreactive) line the slit-like spaces, resembling endothelial cells [38].

Leiomyoma and leiomyosarcoma of the breast show distinct smooth muscle differentiation [39, 40].

Pure rhabdomyosarcoma and osteosarcoma of the breast are composed of cells showing varying degrees of skeletal muscle differentiation or osteoid formation [41, 42].

Periductal stromal tumor is a rare lesion of low-grade sarcoma behavior [43].

The most frequent subtypes of primary lymphomas of the breast are diffuse large B-cell non-Hodgkin lymphomas, not otherwise specified (DLBCL), extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type, and follicular lymphoma. Rare cases of Burkitt lymphoma, lymphoblastic lymphoma of either B-cell or T-cell type, and peripheral T-cell lymphomas have also been reported. Hodgkin and non-Hodgkin lymphomas originating from nodal sites may secondarily involve the breast [44].

Metastasis to the breast represents up to 1.3% of all mammary malignant tumors, including melanoma and carcinomas of the lung, ovary, prostate, kidney, and stomach [45].

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Pathology After Neoadjuvant Treatments

14

W. Fraser Symmans

14.1 Approach and Methods

14.1.1 Pathologic Examination of Post-neoadjuvant Resection Specimens

The main objective is to accurately identify the sites of primary tumor bed (that may or may not contain residual cancer cells) and any residual lymph node metastases, so that the extent of residual cancer can be measured and reported. Of course, the tumor bed can become very subtle after effective neoadjuvant chemotherapy, and improving treatments make this ever more likely. Posttreatment residual disease is often detected by light palpation of the sliced breast specimen even more easily than it is observed by visual inspection. Sometimes that is in an area of ill-defined fibrosis. Fortunately, the communication between pathologists, surgeons, and radiologists has greatly improved and, combined with innovations in preoperative localization, has greatly improved the precision of pathologic evaluation.

Recommendations from an international multidisciplinary committee describe the approach and methods in two publications [1, 2]. For example, a current best standard involves clinical communication through medical records (electronic) to include radiologic reports; placement of metallic clips in each site of primary disease and the biopsy-proven positive lymph node prior to treatment, either a localization wire or radioactive metal seed into the primary tumor and clipped node; and sentinel lymph node biopsy procedure. For example, placement of a radiologic clip in a positive axillary node before treatment increases the accuracy of posttreatment sentinel node biopsy to a clinically acceptable level (false-negative rate of 2%) [3, 4].

Each procedural advance enables pathologists to more accurately identify and evaluate posttreatment specimens. But their effectiveness requires that pathologists have access to specimen radiography to locate metallic clips, coils, seeds, or even calcifications within the specimen and in macroscopic slices of the specimen. Thus, the pathologists can distinguish contiguous from multifocal tumor bed and produce a visual map of the sliced specimen to indicate the sites of the disease and the locations of tissue sampling for histopathologic sections on slides. Indeed, a visual map is critical for accurate pathology, and this can take the form of radiograph, printed digital radiograph, photograph, or sketch (with measurements annotated). The pathologist and technician can draw and label the site of each lesion of interest and each tissue sample for histopathology directly onto the map and then scan that image and save the file with the pathology record. When the pathologist reviews the corresponding slides days later, it will be obvious how any residual disease in the slides relates to the original map. This critical step, although simple, will greatly improve the accuracy of reporting the site, extent, and stage of residual disease. Often times it will also decrease the number of tissue blocks and slides for histopathology, thereby decreasing costs and time as it concentrates the pathologist's attention to the appropriate areas of the specimen.

Typically, an intraoperative evaluation of the primary resection specimen (presence of tumor bed and margins assessment) will be requested. Accurate specimen radiography available to the pathologist expedites this assessment, when combined with gross examination of the sliced specimen, and reduces the need for frozen sections of margins. Intraoperative evaluation of sentinel lymph nodes might also be requested, and this can take the form of touch preparation cytology or frozen section. There are pros and cons to either approach. Each is accurate in practiced hands, and cytology preserves all of the tissue for permanent histopathology sections, whereas frozen section allows for estimation of the size of any metastasis. Molecular approaches to nodal assessment are also possible, but have not been accurately calibrated to estimate the nodal burden of residual disease. If immunohistochemistry for epithelial cells (cytokeratins) is

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to be performed on sentinel nodes, then it is better on the permanent section. This can be helpful to identify remaining tumor cells because cytoreduction can occur in metastases as seen in primary disease.

Taken together, these methods increase the accuracy and efficiency of pathologic evaluation and enable accurate determination of the presence of any residual disease (versus pCR), as well as its 'yp' stage and residual cancer burden (RCB), as described in detail below. Moreover, the most important part of pathologic assessment is the gross pathologic-radiologic correlation and the resultant map of tissue sections that occurs on the day of surgery. Therefore, it is very helpful to familiarize the trainees and support technical staff who handle these specimens as to the objectives of pathologic evaluation posttreatment, so that they understand what is required from their work. Online videos of how actual resection samples are handled can be found linked to the following site: www.mdanderson.org/breastcancer/RCB (Internet search terms: residual cancer burden breast) [5, 6].

14.2 After Neoadjuvant Chemotherapy

14.2.1 Prognostic Importance of Pathologic Complete Response (pCR)

Pathologic complete response (pCR) after neoadjuvant chemotherapy has been recognized since the earliest neoadjuvant trials, to be an excellent response with favorable prognosis. Furthermore, it can be inferred from reading any standard pathology report and easily extracted from case records. Thus, the pCR rate of a chemotherapy treatment is a recognized benchmark of efficacy.

Prescient trials of neoadjuvant chemotherapy demonstrated an increase in pCR rate from the addition of a taxane- to anthracycline-based chemotherapy, also from weekly paclitaxel treatment schedule (versus 3 weekly), and from the addition of HER2-targeted treatment to chemotherapy [7–11]. In each example, a subsequently reported adjuvant trial, sufficiently large for survival analysis, demonstrated improved survival outcomes for the treatment that had increased the pCR rate [12–16]. Indeed, randomized neoadjuvant chemotherapy trials have become accepted for clinical development of new treatments for breast cancer, with pCR as an endpoint for accelerated regulatory approval, but still conditional on demonstrated survival benefit for full approval of the treatment [6]. There is some controversy about the prognostic meaning of observed differences in pCR rate, but greater understanding of pathology, phenotype, and statistical issues will hopefully inform progress [17–20].

One claim that most can agree on is that patients who achieve pCR do have favorable prognosis. Clinical investigators and members of the US Food and Drug Administration collaborated to compare the prognosis of pCR versus residual disease (RD) in a meta-analysis that included the majority of mature multicenter neoadjuvant trials [21]. The meta-analysis clearly demonstrated that pCR afforded improved survival, no matter the treatment received. This result also held in each of the main subtypes of breast cancer defined by hormone receptor and HER2 status, although there was not significant prognostic difference in grade 1–2 HR+/HER2−, and modest prognostic effect in HR+/HER2+ disease [21] and the prognosis of pCR in HER2+ disease did not appear to be as good as for other subtypes [21]. However, two important points must be considered. Firstly, these neoadjuvant trials evaluated the primary endpoint of response retrospectively during an era when the pathology community was not broadly engaged in the clinical trial process, and procedures for localizing treated tumor bed preoperatively and standardized procedures for evaluating post-neoadjuvant specimens were very uncommon. Secondly, the follow-up was insufficient to observe long-term prognosis, since fewer than 10% of subjects were remaining at risk beyond 5 years of follow-up [22]. Nonetheless, this meta-analysis was critically important to demonstrate the global experience that pCR is a surrogate endpoint for better prognosis [21].

14.2.2 Prognostic Tools to Categorize Prognostic Risk of Residual Disease

There are two main prognostic tools based on an estimate of the extent of residual disease: AJCC stage ('yp' stage categories) and residual cancer burden (RCB). There are also other tools that combine the assessments of residual disease with information about the disease before treatment began: Miller-Payne system and the Neo-Bioscore (CPSEG) categories. Other systems also exist, but are used less commonly.

14.2.2.1 Stage

The AJCC staging system after neoadjuvant chemotherapy ('yp' stage) is generally similar to the usual 'p' stage of an untreated resected breast cancer, except that it accounts for cytoreduction of a tumor bed at the primary or metastatic site by requiring that the tumor size be the largest residual focus of invasive cancer—without intervening fibrosis. The new 8th edition retains this same definition for measurement when determining ypT and ypN stage (in press). However, it is unusual to have residual primary tumor without intervening fibrosis between nests or masses of cancer cells, and pathologists' assessment of ypT size may vary based on this interpretation. Nonetheless, the categories of 'yp' stage are generally prognostic in breast cancer and within the major subtypes [23, 24].

14.2.2.2 RCB

The residual cancer burden (RCB) is an index score that is derived from the two-dimensional measurements of the extent of residual primary invasive cancer, the histopathologic estimate of the proportion of that area that contains residual invasive cancer, the number of positive lymph nodes, and the size of the largest metastasis (Table 14.1). These variables are entered into an online website to calculate the score and resultant category. RCB scores are prognostic, independent of 'yp' stage and pretreatment 'c' stage, and exhibit good reproducibility between pathologists [5, 25]. So too, the distribution of RCB index scores for residual disease is also classified as minimal, moderate, and extensive RCB (i.e., RCB-I, RCB-II, and RCB-III), and those RCB classes are prognostic [5]. The RCB system has web-based protocols, examples, and educational videos, in addition to the calculator: www.mdanderson.org/breastcancer/RCB.

There are important differences between the 'yp' stage and RCB system, particularly with respect to the measurements recorded for primary tumor and lymph nodes. Tumor size for ypT includes the largest continuous focus of residual invasive cancer, without fibrosis. On the other hand, RCB requires measurement of the extent of the residual invasive cancer related to the tumor bed. This allows for multiple foci of residual cancer in a tumor bed to be included as one overall tumor measurement. Of course, the estimate of cellularity in that tumor area will correct for low cellularity or tracts of fibrosis separating cellular foci. Therefore, we would expect that largest dimension for RCB can be larger than the ypT size, since many tumors regress in a nonuniform way (Fig. 14.1).

The definition of metastatic size in nodes (e.g., to determine macro- versus micrometastasis or isolated tumor cells) for ypN stage is also based on the largest contiguous tumor focus. On the other hand, the size of the largest metastasis for RCB allows intervening fibrosis within a residual metastasis. So again, the metastasis size for RCB can be larger than the size determination for ypN stage, since residual metastases can have nonuniform distribution of cancer cells within the metastatic site.

Lymphovascular emboli are sometimes the only identifiable residual primary cancer, and this can lead to confusion. Firstly, it should be distinguished from intraductal cancer. Secondly, it should be considered to be residual invasive disease. The staging system does not directly address this issue, but the case should not be defined as pCR. The RCB system considers this to be invasive disease and recommends the extent be estimated as if it were invasive disease. This can be problematic if there are only rare isolated foci scattered in the breast. In that setting, it can be reasonable to use the largest focus. But if there are multiple foci, then it is better to estimate the overall area involved and a very low percent cellularity in that area.

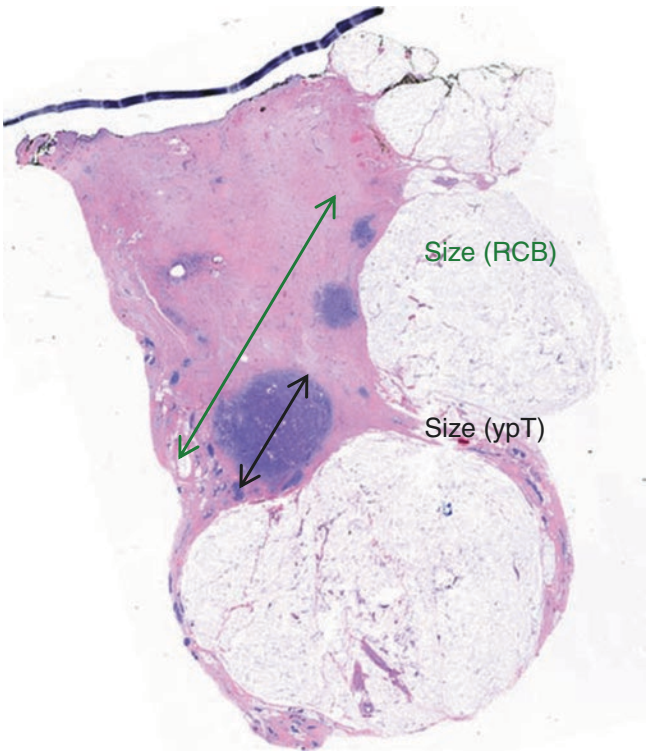

Fig. 14.1 Illustration of the different criteria for measuring residual invasive cancer size for ypT versus extent of residual invasive cancer for calculation of RCB. This same difference in approach also applies to the measurement of nodal metastases

The most common challenges for pathologists using the RCB system are (1) failure to realize that the primary tumor bed area for RCB is defined from the combined information from gross examination of the specimen and subsequent histopathologic study of the corresponding slides and (2) overestimation of the average cancer cellularity within the area of the primary tumor bed. As with any resected tumor, the macroscopic measurements are preliminary results that may be revised after review of the corresponding microscopic findings. Thus, the tumor bed for final measurements in the RCB formula and for assessment of cancer cellularity is ultimately defined from the histopathologic assessment (Fig. 14.2). The RCB website contains pictures and videos to prime pathologists as to how to assess average cellularity across an area, and these are helpful to avoid the mistake of taking cellularity estimates only within concentrated foci of cancer cells in the tumor area—since that approach would not provide the average cellularity. Hence, the average cellularity of residual invasive cancer in the tumor bed for RCB can be lower than the cellularity of the most concentrated tumor focus that one might record for genomic testing, and it represents cancer cellularity per area rather than cancer cellularity per nucleus.

Overall, the RCB has been independently validated, provides prognostic information that is independent of 'yp' stage, and is relevant within each phenotypic subtype

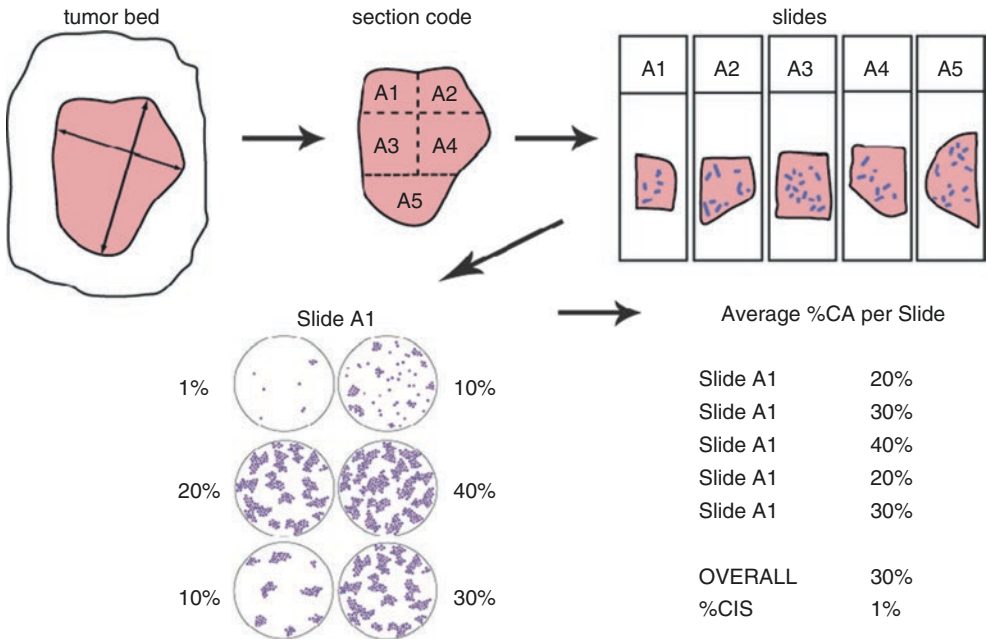

Fig. 14.2 Schema for mapping gross and histopathologic findings from the largest residual primary tumor bed to define the dimensions and average cellularity of the residual invasive tumor bed for calculation of RCB

(reported in abstract by Symmans et al. San Antonio Breast Cancer Symposium 2013, manuscript under review). Its greatest potential for use within clinical trials has barely been tested: to compare the distributions of RCB index scores between experimental and control populations from a randomized trial. That would enable every participant in the trial to contribute relevant information about response and prognosis.

14.2.2.3 Miller-Payne

The Miller-Payne system compares the cellularity of the residual invasive cancer to the cellularity of the cancer in the pretreatment core biopsy. The five categories of relative cytoreduction are prognostic [26]. Although residual tumor size and nodal status are not considered, there is correlation between lesser stage and cytoreduction [27]. This system has not been evaluated compared to stage or within the phenotypic subtypes of breast cancer.

14.2.2.4 Neo-Bioscore (CPSEG)

The CPSEG system is a nomogram that adds risk integer scores (0–2) for the following individual criteria: clinical stage (cT, cN) before treatment, pathologic stage after treatment (ypT, ypN), estrogen receptor status, and histopathologic grade before treatment, to produce 7 prognostic groups (Table 14.2) [28]. This system has been independently validated as prognostic [29, 30]. Recently, the system was revised to include HER2-positive status as a favorable prognostic criterion because response and survival has been improved by addition of HER2-targeted therapy to chemotherapy and renamed as Neo-Bioscore [31]. The advantages of CPSEG/Neo-Bioscore are that the system uses existing standard data and is readily accessible to retrospective databases. However, it is broadly relevant to breast cancer, and the prognostic meaning of some of these variables included in Neo-Bioscore might vary according to the subtype of disease—particularly as practice approaches to neoadjuvant therapy and types of treatment used are becoming more subtype specific. Indeed, the subtype specificity and applicability to new treatment paradigms will be a challenge for all of the prognostic tools.

14.2.3 Cellular and Molecular Characteristics of Residual Disease

It would seem likely that the biological characteristics of residual disease would add clinically relevant information to the extent of residual disease. For example, response (RCB) and predicted endocrine sensitivity (SET index) appear to independently influence the prognosis after sequential neoadjuvant chemotherapy followed by adjuvant endocrine therapy, possibly with interactive synergy [32]. Also, the residual proliferation index after chemotherapy can add to the prognosis of RCB [33]. Also, the presence of abundant lymphocytes associated with residual cancer imparts a more favorable prognosis when there is more than minimal residual disease [34]. Descriptive studies have also identified mutations within posttreatment residual disease (e.g., *JAK2* mutations in triple-negative cancer) [35, 36]. So there is some precedent to pursue these associations further. However, one does need to be aware that low cellularity can influence the accuracy of molecular testing. The key questions for progress will be

to identify when additional cellular or molecular characteristics of residual disease add meaningful information to the extent of disease, to avoid strongly correlated variables, and to specifically identify phenotype-specific indications for this approach.

Another consideration is whether to repeat routine molecular tests on residual disease. Certainly, a reasonable case can be made to repeat hormone receptor and HER2 tests if the original results before treatment were negative and there is reasonable suspicion that there might be positive receptor status. One can argue that the potential benefit from adjuvant treatment, when a false-negative original result is corrected, far outweighs the expense of repeat testing—so long as clinical-pathologic judgment is used to select cases where the overall picture raises suspicion for false-negative result. On the other hand, there is at least one study describing worse prognosis when the residual disease turned HER2 negative after neoadjuvant chemotherapy with HER2-targeted therapy [37]. However, there is no clinical justification for withholding adjuvant HER2-targeted therapy from these patients. Generally, retesting should be considered when original results were negative and a false-negative result is possible, but otherwise not.

14.3 After Neoadjuvant Endocrine Therapy

Neoadjuvant endocrine therapy is considerably less common than chemotherapy, but there are several trials that have utilized this approach, and several conclusions can be made based on current knowledge. The principles of specimen evaluation (described above) apply equally to any post-neoadjuvant specimen. Unfortunately, clinical trials have not directly compared different prognostic pathology tools (described above) after months of neoadjuvant endocrine therapy, although it might be reasonable to assume that those tools would be informative. However, the main finding from studies to date is that pharmacodynamic suppression of proliferation (e.g., Ki67 immunostain) after exposure to endocrine therapy is a hallmark of tumors that have sensitivity to endocrine therapy. Consequently, the preoperative endocrine therapy prognostic index (PEPI score) is currently recommended as the best prognostic tool for pathologists to use after neoadjuvant endocrine therapy [38]. PEPI is a nomogram that adds the scores for posttreatment ypT, ypN, ER status, and the percent residual cancer cells that express Ki67 (Table 14.3). The total score (0–12) is summarized into three prognostic groups: PEPI score 0, PEPI scores 1–3, and PEPI scores ≥4 [38]. There has not been independent validation of prognostic validity of PEPI yet, but we expect that those results will be reported soon.

14.4 Novel Treatments

It is reasonable to expect that the principles of specimen evaluation after neoadjuvant treatment will apply to novel treatments and that additional biological information might become useful. However, one cannot assume that the definitions of response won't improve or become specific to classes of treatment or that differences in response rates will necessarily predict differences in survival. We have already observed this: the prognosis relating to residual disease is influenced by sensitivity to other adjuvant treatments after surgery in relevant subtypes of breast cancer [32]. So the efficacy of novel treatments can be assessed within the neoadjuvant model, and we can also learn of the effects on tumors, but fundamental principles must still apply: prospective clinical trials, randomization, and obtaining subsequent follow-up for survival.

Some novel treatments are being evaluated as adjuvant treatment after neoadjuvant treatment and surgery, wherein patients become eligible based on an estimate of their residual prognostic risk. That is largely defined by the extent of residual cancer and the disease subtype. Such studies should not naively expose patients with minimal residual disease to the risks of experimental treatments, if the defined minimal disease has excellent prognosis. Also, such studies should collect detailed pathology information from standardized pathology methods, so that the investigators can determine whether pathology findings after neoadjuvant treatment can identify the patients who would benefit from the post-neoadjuvant novel treatment.

14.5 Summary

Neoadjuvant treatment has become increasingly popular as a standard treatment, and in clinical trials, it becomes increasingly obvious that the prognostic pathologic information from residual disease outweighs the information from untreated disease. However, pathologists may be unaware that a patient received neoadjuvant treatment, ill-equipped to identify and map the extent of residual disease, have limited experience with such specimens, or simply be reluctant to change their practice to support clinical trials. Thus, global standards are needed to the methods of pathology evaluation of specimens after neoadjuvant treatment. Indeed, an international committee, representing clinical trial groups, already recommended that all cases should report the necessary information to report the following after neoadjuvant chemotherapy:

1. pCR (ypT0/is, ypN0 or ypT0, ypN0) versus residual disease
2. AJCC stage (ypT, ypN)
3. residual cancer burden (RCB) [1, 2]

If neoadjuvant endocrine therapy was administered, then PEPI index should also be reported. These information provide important prognostic information, and pathologists should adjust their practice to report them for clinical trials and routinely. Furthermore, use of a standardized method for pathologic evaluation is reproducible, adds no cost to patient care, and is usually more efficient of time and costs. However, the accuracy and efficiency of this approach does require modest support from hospitals to enable specimen radiography and to provide resources to image and create a map of where tissue sections relate to macroscopic findings in the specimen, and that also requires the support from the multidisciplinary breast cancer team to garner the necessary resources. Finally, posttreatment residual disease should sometimes be retested for standard molecular markers, if there is reasonable suspicion that the pretreatment result might have been a false-negative result, or to evaluate pharmacodynamics changes in levels of ER or Ki67 after neoadjuvant endocrine therapy.

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Breast Cancer Genomics

15

Maurizio Scaltriti

15.1 Introduction

Recent large-scale next-generation sequencing (NGS) studies defined the landscape of genomic aberrations in breast cancer. Copy number variations, missense mutations, and small insertion/deletions of certain genes can be associated with carcinogenesis and tumor progression. These so-called cancer drivers supposedly confer either growth advantages or protection from therapeutic stress.

Some of these genomic alterations can be inherited, but the vast majority occurs in somatic cells by stochastic events in DNA editing/repair and environmental mutagens.

In primary breast tumors, the most frequently altered genes are *TP53*, *PIK3CA*, *MYC*, *CCND1*, *PTEN*, *ERBB2*, *ZNF703/FGFR1* locus, *GATA3*, *RB1*, and *MAP3K1* [1]. This scenario, however, can change in the metastatic setting and/or in tumors subjected to pharmacological pressure. An archetypal example of this divergence is the mutation rate of *ESR1*, the gene encoding for estrogen receptor alpha (Fig. 15.1). While mutations in this gene are found in less than 1% of primary tumors, up to one-third of patients relapsing from anti-endocrine therapy have tumors harboring *ESR1* mutations [2].

Adding another layer of complexity, it is now possible to use mathematical approaches to define mutational signatures associated to specific genomic rearrangements, gene expression patterns, or clinical features [3, 4]. Although these signatures may define more precisely the genomic status of the tumors and can provide some prognostic information, their exploitability in the clinic is debatable.

In this chapter, I will focus on the possibilities that practice oncologists currently have to offer rational therapeutic options based on genomic analysis.

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15.2 Change in Mentality

It is generally thought that genomic testing is appropriate only for patients who have exhausted standard therapy or have orphan tumors that lack a standard therapy. However, I contend that with the current possibilities of analyzing tumor samples by targeted exome sequencing (see below), each patient with advanced disease or with high-risk prognostic situations should at least be given the opportunity of being informed about genomic approaches other than following the standard protocols. Not having genomic infrastructures or not being a recruiting center for clinical trials is not a valid argument to refute this possibility. As I will describe below, anybody can easily submit ordinary formalin-fixed paraffin-embedded (FFPE) samples to certified companies that can sequence the tumor DNA in matter of weeks. Although the cost of this procedure can be burdensome, it may be affordable for the majority of the patients.

Patients should at least be informed about this possibility, explaining very clearly that this analysis will not provide a "magic pill," but it can potentially uncover genomic vulnerabilities in the tumor, and in turn help in choosing the specific targeted therapies. Obviously, the oncologist also has to explain that, should this be the case, the matching therapeutic option may be either available as standard of care or, conversely, part of experimental studies that are not necessarily available in the same geographic area. The patient has to decide whether undertaking this path is worthwhile, with very little influence from the treating physician.

No real advances in medicine (or in science in general) have ever been made by just following the existing paradigms and not thinking outside the box. The word "conventional" in medical care is a very dangerous one. Without reaching "Newtonian" extremes, I believe there is a need for a radical change in the way cancer patients are managed and treated. The gap between the first lines of research and the current clinical practice is too wide and too often is seen as the difference between science fiction and the real world. This has to change.

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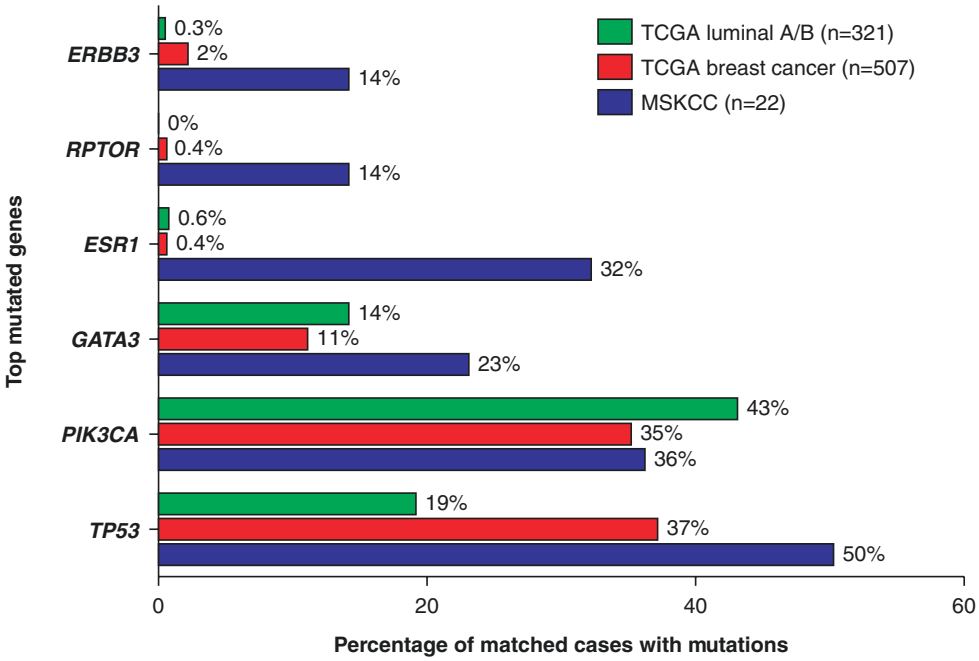

Fig. 15.1 Different prevalence of mutations between tumors relapsed to anti-endocrine therapy (MSKCC cohort) and tumors from the TCGA

15.3 What We Can Realistically Do

The genome of the tumors can be analyzed in several ways. Whole genome sequencing (WGS) provides a detailed map of single nucleotide variations (SNVs), insertions and deletions (indels), gene translocations, and copy number changes. WGS is certainly the most complete approach, but has a number of important caveats. It generates an enormous amount of information, but most of it is of unknown clinical importance. It requires a relatively high amount of tissue, not always available from tumor biopsies. Moreover, besides the still prohibitive costs for its standard application in the clinical setting, WGS entails complex informatics analysis and big data storage. Whole exome sequencing (WES) provides the same map of genomic aberrations (with the exception of gene translocations) present in the genes that are expressed in mRNA. WES requires less material and cost is lower compared to WGS, but, although the amount of information generated is also reduced, it still involves a level of analytical work not wanted (and not needed) in the clinical practice. A more targeted approach to sequence the genome seems to be more reasonable for the widespread clinical implementation of this technology.

The field started with the detection of single gene mutations that allowed some patient stratification for certain therapies (e.g., KRAS detection in colon cancer) or the detection of germinal alterations linked to increased risk of developing cancer (e.g., BRCA1/BRCA2 mutations in both ovarian and breast cancer). Subsequent advances included the detection of well-known gene mutations (so-called hotspots) associated with tumor onset and/or resistance to therapy. Although merely diagnostic, these tests were useful to better stratify patients for clinical trials testing novel targeted agents.

The real revolution in the field was the development of targeted (or capture-based) exome sequencing platforms, in both research and clinical settings. From ~200 ng of DNA or less it is now possible to gather genomic information that is easily interpretable and can strongly influence the practice of treating medical oncologists. This technology does not require specific sample preparation and is commercially available and usually friendly to order online. Samples can be shipped at room temperature, and results are returned in the form of an easily interpretable report in 4–6 weeks.

This approach ensures deep exome sequencing (high-gene coverage) of a selected number of genes (usually a few hundred) considered important for tumor progression and resistance to therapy. A comprehensive analysis of the coding sequences of these genes may inform on the intrinsic genomic vulnerabilities of the tumor and therefore its possible sensitivity toward a given targeted therapy (e.g., *PIK3CA* mutations for PI3Kα inhibitors or *BRCA1/BRCA2* mutations for PARP inhibitors). Moreover, targeted exome sequencing can in some instances inform on the genomic instability of the tumor, perhaps rendering it more likely to respond to DNA-damaging agents or provide prognostic information (e.g., *TP53* mutations). Moreover, thanks to the quantification of the allele frequencies of each mutated genes, it can reveal the presence of different subclonal populations within the tumors and roughly estimate the level of tumor heterogeneity. Similarly, targeted exome sequencing can estimate the mutational load of the tumor, based on the number of driver and passenger mutations, especially when associated with mutations of genes involved in DNA repair.

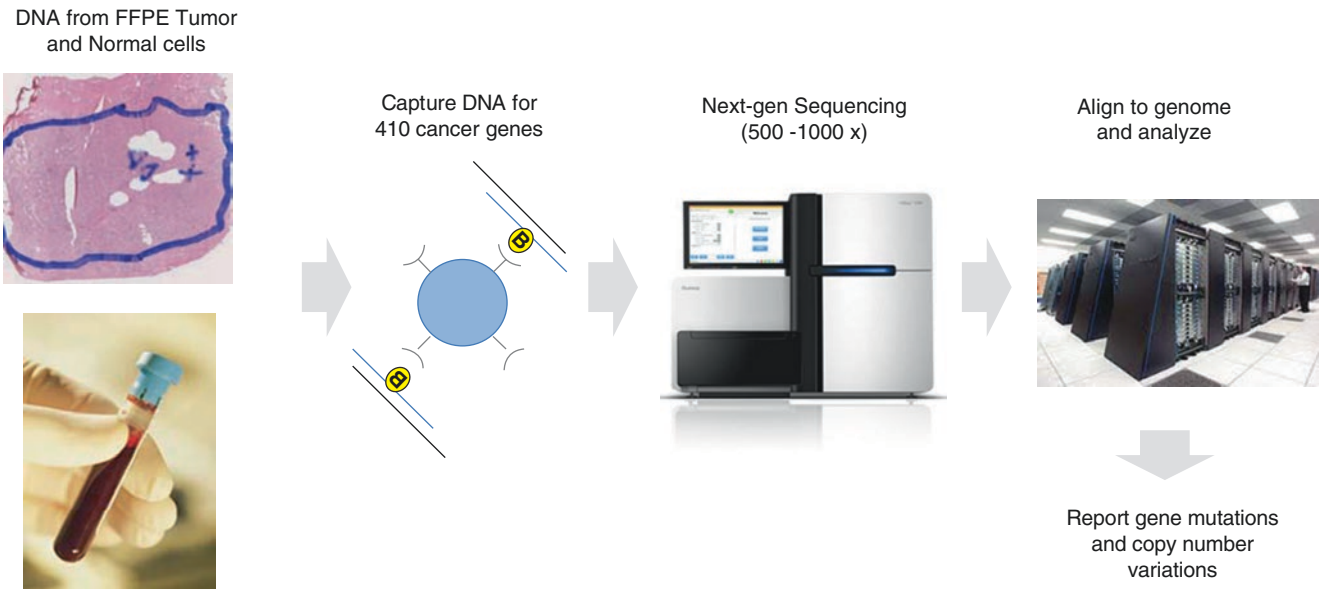

Fig. 15.2 MSK-IMPACT targeted exome sequencing platform. DNA is extracted from either tissue or blood and only protein-coding exons of 410 cancer-associated genes are sequenced

Not ignorable is also the possibility to uncover possible not-so-obvious drivers present at a relatively low frequency. Two valid examples in breast cancer are HER2 and AKT1 mutations, which strongly predict for response to pan-HER kinase inhibitor neratinib [5] and AKT kinase inhibitors [6], respectively.

In addition to Clinical Laboratory Improvement Amendments (CLIA)-certified companies that provide this service, several cancer centers worldwide have developed their in-house platforms. One of the most successful examples is the MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a targeted exome sequencing platform developed at Memorial Sloan Kettering Cancer Center (MSKCC) [7, 8]. The MSK-IMPACT platform has a track record of performing in small FFPE biopsies and cell blocks and ensures a deep sequencing coverage of 410 key cancer-associated genes (Fig. 15.2). To date, more than 10,000 patients (more than 1000 breast cancer patients) had their tumor sequenced with this platform at MSKCC, with ~60% of the cases having metastatic disease. Thanks to this effort, an unprecedented percentage of patients are entering in clinical trials and being treated with rationale-based targeted therapies. Some of these patients are experiencing exceptional responses unlikely to be seen with "canonical" therapies.

The accumulation of sequencing reports from breast cancer patients also allows identifying novel correlations between certain genomic alterations and sensitivity to given therapy. Moreover, this high number of cases permits to uncover new hotspot mutations and/or new drivers of disease.

15.4 Tumor Heterogeneity

Most tumors are thought to originate from a parental clone that accumulates genetic aberrations during carcinogenesis. As a result, these aberrations are present in the majority of, if not all, tumor cells in the neoplasm. During tumor progression, however, spatially distinct subclones characterized by heterogeneous somatic mutations and chromosomal imbalances may arise from the parental clone [9]. The aberrations that arise during subclonal evolution are thought to be the result of the selective pressure exerted by the tumor environment and/or as an adaptive response to antitumor therapy.

It is widely accepted that the sensitivity to a given drug depends on tumor heterogeneity. The proportion of tumor cells that express the target of interest or harbor the mutation determinant of sensitivity may vary among the different metastatic sites (inter-tumor heterogeneity) or within single lesions (intra-tumor heterogeneity). Therefore, tumor heterogeneity can predict the degree of drug sensitivity and possibly the selection of resistant clones.

Such heterogeneity of subclones within the primary tumor or metastases represents a major challenge in the management of cancer for a number of reasons. Tumor biopsies are invasive procedures and may be associated with complications [10] and significant costs. Additionally, a single biopsy may be subject to tumor sampling bias and thus fail to capture the therapeutically relevant mutations [9, 11, 12]. Furthermore, in those tumors with several metastatic sites, the sampling bias is surely amplified as there is well-described genomic branching of the tumors [9, 11–15]. As a

result, intra-tumoral heterogeneity may explain the difficulties encountered in the validation of oncology biomarkers and for prediction of therapeutic resistance.

A number of studies have shown that ctDNA may be isolated from blood [16–18]. Early studies of ctDNA demonstrated that polymerase chain reaction (PCR)-based assays could accurately detect the mutations that have already been identified in the tumor bulk [19–24]. In preliminary studies it has been shown that targeted and genome-wide NGS of ctDNA is a feasible approach and can be employed to identify genomic alterations in the ctDNA [20, 25–30]. NGS ctDNA assays can potentially provide an easily obtainable and minimally invasive surrogate for tumor tissue biopsies that will markedly facilitate identifying potential targets to guide treatment decisions. It may also provide a potentially sensitive and specific biomarker that can be monitored in real time during therapy.

Collectively, understanding the extent to which the genetic heterogeneity among subclonal populations in the same patient converges to a similar and targetable phenotype may contribute to more rationale-based therapeutic approaches.

15.5 Sequencing to Understand Drug Resistance

Resistance to therapy can be pre-existing (intrinsic) due to the presence of concurrent aberrations. In breast cancer the presence of certain genomic aberrations can predict the response to given therapeutic agents. HER2 amplification, for example, is a determinant of sensitivity to drugs such as trastuzumab, pertuzumab, lapatinib, or trastuzumab emtansine (T-DM1). The presence of *PIK3CA*-activating mutations, on the contrary, is associated with resistance to these agents (with the exception of T-DM1 [31]). However, harboring these *PIK3CA* mutations is required to respond to PI3K p110α inhibitors [32, 33].

More frequently, drug resistance can arise upon therapeutic pressure via either loss of the therapeutic target [34] or positive selection of resistant clones [35, 36]. In fact, the constant pharmacological pressure may favor the fitness of tumor cells harboring certain genomic features and result in acquisition of drug resistance.

This occurrence is very well known, for example, in EGFR-mutant lung cancer, where the acquisition of the T790M gatekeeper mutation in EGFR or the amplification of other receptor tyrosine kinases such as MET or HER2 represents the majority of the mechanisms of resistance to the anti-EGFR molecule erlotinib. In breast cancer, several genomic mechanisms of acquired therapy resistance have been recently validated in the clinic. The selection of cells harboring *ESR1* mutations following endocrine therapy in ER-positive tumors is perhaps the most obvious example [2]. There are also evidences that the regeneration of a functional *BRCA2* upon therapy with PARP inhibitors leads to emergence of drug resistance [37]. Another example is the discovery of parallel genomic evolution occurring in patients treated with a PI3K p110α inhibitor where PTEN expression was lost over time via six different genetic mechanisms [12]. In this work, we discovered that different genomic aberrations but leading to the same convergent resistance phenotype can coexist in different metastatic lesions. The tumor genomic evolution during pharmacological stress was studied in a patient with metastatic breast cancer treated with the PI3Kα inhibitor BYL719 achieving a lasting clinical response, after which drug resistance emerged and died shortly thereafter. A rapid autopsy was performed and a total of 14 metastatic sites were collected and sequenced. When compared to the pretreatment tumor, all metastatic lesions had a copy loss of *PTEN*, and those lesions that became refractory to PI3Kα inhibition had additional and different *PTEN* genetic alterations (either copy number loss or missense mutations), resulting in the loss of PTEN expression (Fig. 15.3). Acquired biallelic loss of *PTEN* was found in one additional patient treated with BYL719, whereas in two patients *PIK3CA* mutations present in the primary tumor were no longer detected at the time of progression. These findings were functionally characterized in the laboratory using both in vitro and in vivo preclinical models that confirmed the causative role of *PTEN* knockdown in inducing resistance to PI3Kα inhibition.

This work is an archetypical example that access to metastatic lesions of patients who initially responded and then progressed to a given targeted therapy is crucial to elucidate the role of tumor heterogeneity and genetic evolution in the acquisition of drug resistance. Moreover, it also underscores the power of rapid autopsies of particularly informative patients (e.g., exceptional responders—see below) in uncovering novel genomic aberrations associated with drug sensitivity.

Without relying on these extreme cases, however, it is reasonable considering to re-biopsy at disease progression for genomic sequencing. In addition to confirm the origin, morphology, and the HER2/ER status of the tumor, this practice can inform of genomic vulnerabilities lost or gained during the treatment. This is of pivotal importance as it may uncover mechanisms of acquired resistance to therapy and set the stage for the next therapeutic option.

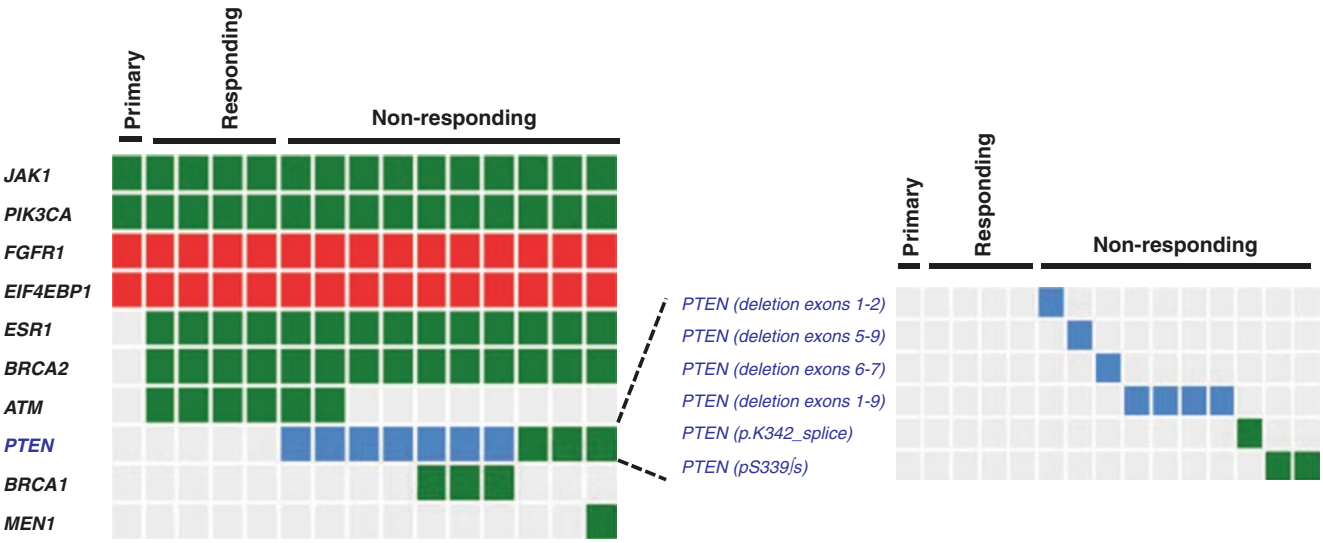

Fig. 15.3 Convergent evolution resulting in genomic loss of *PTEN* in metastatic lesions from a breast cancer patient treated with a selective PI3Kα inhibitor

15.6 Exceptional Responders

Among the population of patients treated with either chemotherapy of targeted agents, there are rare cases that, unpredictably, show much higher-than-expected (or lower-than-expected) clinical responses. Examples of exceptional responders can be HER2-positive metastatic breast cancer patients that respond to trastuzumab-based therapy for a decade or show durable pathological complete response to trastuzumab without concomitant chemotherapy [38] or triple-negative breast cancer patients with metastatic disease that show durable response to cytotoxic chemotherapy. But perhaps the best examples are those patients that already progressed to every standard of care options but still achieve dramatic and/or durable response to investigational agents.

The tumors from these patients are likely to have particular molecular/genomic characteristics that render them exquisitely sensitive to the therapy. Samples obtained from patients catalogued as exceptional responders (or exceptional resistant) represent an invaluable source of material to study the intrinsic determinants of drug sensitivity and/or tumor evolution upon therapeutic pressure. These samples should be analyzed as thoroughly as possible, with all the available resources. In some of these cases, oncologists chose (wisely) to collaborate with academic centers that have the possibility to perform both WES and WGS. Besides mutations or changes in gene copy numbers, these tumors may harbor gene rearrangements or alterations in noncoding DNA (e.g., gene promoters, enhancers). If tissue procurement is not a limiting factor (i.e., surgical removal of the primary tumor or distant metastases), RNA sequencing or other platforms that consent to evaluate the gene expression profile of these lesions are strongly advised. Results from these analyses can potentially identify genomic or transcriptomic markers of response (or resistance) that, if confirmed in larger cohort of patients, will allow a more rational patient stratification.

Unfortunately, also exceptional responders may recur to therapy and develop drug resistance. As mentioned above (and if the onset of a different tumor is discarded), these lesions are likely to be the results of a selection of cells bearing genomic aberrations that confer fitness under the pharmacological pressure. Thus, they should be analyzed as deeply as possible and confront their genomic landscape with the one of the matched therapy-sensitive tumors. Each individual case may indicate possible mechanisms of drug resistance that can be confirmed using publicly available data and/or by testing the hypotheses in the laboratory.

15.7 Therapeutic Plan of Action

Once we have gathered the sequencing data of our tumor samples, we need to interpret the results and explore possible therapeutic options. The ability to identify patients with tumors harboring specific genomic vulnerabilities and match

them to the most appropriate therapy is central. At least four different scenarios may be in front of us:

1. We discover genomic aberrations that are targetable and for which there are FDA-approved drugs for that indication. This is obviously the best possible situation.
2. The genomic alterations present in our tumor sample indicate that one or more FDA-approved agents may be effective in this particular case but these agents are not registered for their use in breast cancer patients. In these circumstances it is generally feasible to ask for compassionate use of these drugs. This requires some extra work and ultimately depends on the positive response of the pharmaceutical companies, but it would be unethical not to attempt it.
3. The sequencing results uncover actionable mutations or gene amplifications that would justify the inclusion of the patient into an existing clinical trial testing the activity of a compound still under investigation. This scenario is likely the major deterrent for the broad use of genomic testing as routine diagnostic practice. As a matter of fact, one common argument made against the genomic characterization of tumor samples is the lack of available clinical trials in the geographic area of interest. In other words, the oncologist may wonder why the patient should be sequenced if there are no options to offer a therapeutic strategy based on the genomic data. This point deserves some considerations. First of all, it is very unlikely that every oncologist is aware of every clinical trial open and enrolling at a given time in their geographic area. Secondly, the term "geographic area" is very subjective. Some patients may be intimidated of traveling hundreds of kilometers or even consider a clinical study abroad, but some others may think that the chance of receiving a rationale-based therapy is worth the hassle.
4. The last scenario is the most frustrating. Actionable genomic aberrations are identified, but only experimental drugs under investigation for other tumor types could potentially be used to achieve clinical benefits.

Despite these premises, it is undeniable that a major limitation in developing precision medicine approaches is the fact that only few cancer drivers are represented at a relatively high frequency. As a matter of fact, we calculate that 85% of all hotspot mutations affect <5% of any cancer type in which they are found [39]. This problem can be partially overcome by enrolling patients based on genomic alterations rather than tumor type, following the concept of the "basket" clinical trial. This formula consents to test investigational drugs to a variegated patient population harboring the same genomic aberration. An example is the recently published results from patients with BRAF-mutant solid tumors treated with the small molecule BRAF inhibitor vemurafenib [40]. In this study, they found that the BRAF V600 mutation is a targetable oncogene in several cancer types other than melanoma. Dramatic responses were observed in cancers that would have had no therapeutic options if the patients were not part of this study. A patient with a rare case of BRAF-mutant breast cancer, for example, could be treated with vemurafenib by either compassionate use or by being enrolled in such trial (scenario n. 2). Similarly, breast tumors with a *NTRK* fusion (rare but often extremely dependent on this gene translocation) could be treated with NTRK inhibitors as part of an existing basket trial testing these compounds (scenario n. 3 or 4).

Other examples are the ongoing clinical trials testing the activity of the pan-HER inhibitor neratinib and the AKT inhibitor AZD5363 in HER2-mutant and AKT1-mutant tumors, respectively. Recent large-scale NGS studies have revealed recurrent activating *ERBB2* (the gene-encoding HER2) mutations across a wide variety of cancer types. In breast cancer, these activating mutations are relatively rare (~2%) and typically mutually exclusive with amplification of the gene [41]. These patients, therefore, are excluded from receiving conventional anti-HER2 therapy. Similarly, *AKT1* E17K mutations arise in ~3% of breast cancers, and despite compelling preclinical data that supports a central role for oncogenic AKT1 in the pathogenesis of many cancers, it remains unknown whether mutant *AKT1* is a rational therapeutic target. In both cases, heavily pretreated metastatic breast cancer patients are experiencing impressive responses to inhibitors of these kinases are given in combination with the ER degrader fulvestrant. These studies are currently being carried out only in selected institutions, but they represent proofs of concept that targeted therapies based on genetic characterization may provide clinical benefit even in patients that exhausted any other therapeutic options. The long-term objective is to expand these studies to more hospitals and bring these targeted agents to early-phase treatments in selected patient populations.

A parallel approach to predict the most effective therapy based on genetic data is the use of patient-derived xenografts (PDXs) harboring the same genomic aberrations found in the analyzed tumor. In some cases the xenograft model may be derived from the same tumor lesion that has been sequenced. Although this practice is widely used in translational research in cancer centers via academic collaboration, it is not as diffused in the clinical practice. It is, however, possible to ship fresh tumor samples to specialized companies that can provide this service. The use of PDXs is particularly useful in those cases where the rationale for the use of a given drug is not very strong or there are multiple therapeutic options available.

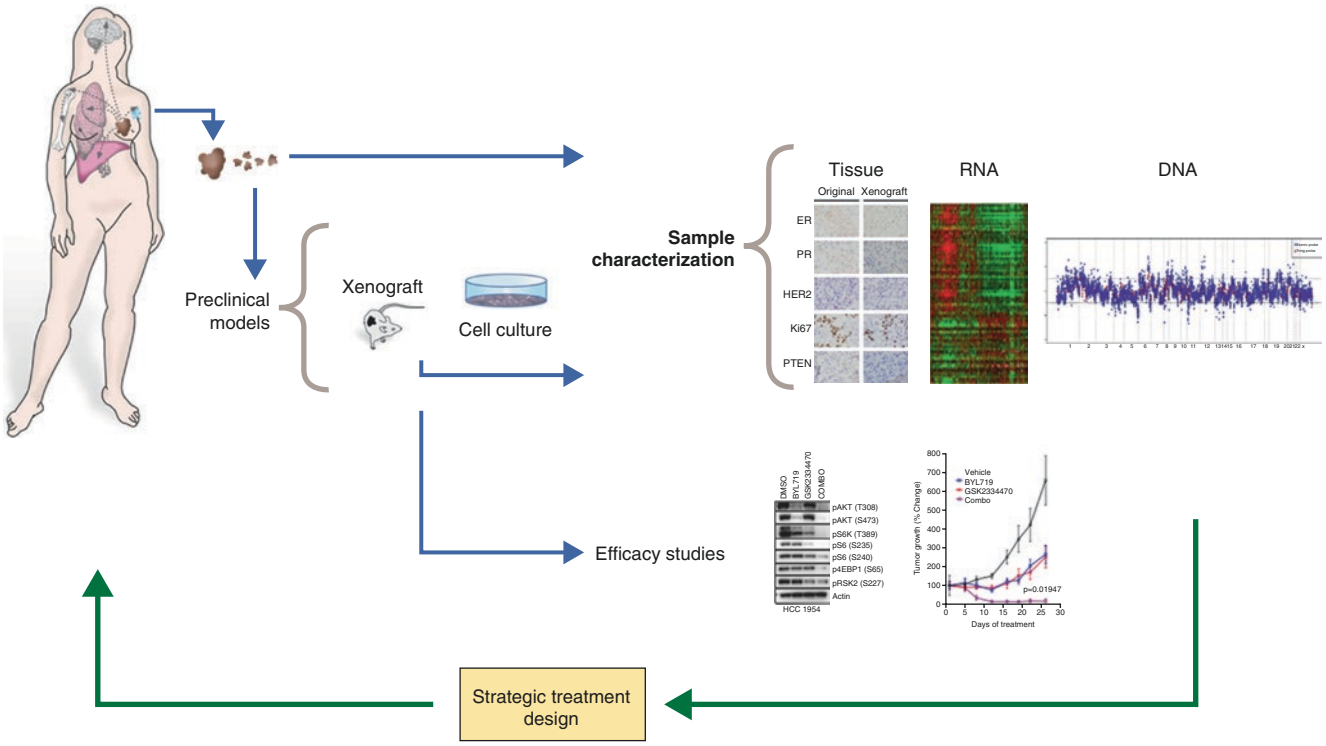

Fig. 15.4 Personalized medicine approach. Tissue from either primary tumors or metastatic lesions is characterized by canonical techniques and next-generation sequencing. In parallel, preclinical models from the same samples can be established to test rationale-based therapeutic options

Conclusions

Increased accessibility to targeted platforms and the expansion of clinical studies that enroll patients based on the genetic vulnerabilities will be key to move toward personalized medicine. An interesting pilot experiment to increase the number of patients undergoing genetic testing followed by matched targeted therapy is the Spanish study AGATA (NCT02445482). To be enrolled in this study, patients belonging to a defined geographic area have to consent that a committee of both clinical and translational investigators will suggest their enrollment in the most appropriate clinical studies based on their sequencing data. In principle, the more participating centers, the more available clinical trials that can recruit these patients. Moreover, this approach may speed the accrual of many of these studies with patients that, supposedly, are more likely to respond to the investigational therapies.

Another benefit of genomic sequencing that should not be underestimated is the detection of gene aberrations predictive of lack of response to given therapies. Perhaps the genomic results will not identify an actionable gene or pathway, but will indicate which compound should not be chosen for that patient, avoiding the toxicity and economic burden of treatments that will most likely fail. KRAS mutations, for example, are well known to limit the sensitivity to cetuximab in colon cancer. In breast cancer, the presence of *ESR1* mutations is indicative of resistance to tamoxifen and/or aromatase inhibitors [2], the loss of *PTEN* is sufficient to discourage the therapy with specific PI3Kα inhibitors [12], and the loss of *RB* renders CDK4/6 inhibitors ineffective [42].

It is tempting to imagine that soon every breast cancer patient will have their tumor DNA sequenced, perhaps multiple times, in order to monitor disease progression, thus enabling a rational use of molecularly guided therapies (Fig. 15.4). Similar to the antibiogram that is normally done for bacterial infection to choose the most effective antibiotic, the genomic aberrations of each tumor may one day be used to routinely indicate the most appropriate antitumor therapy for each patient.

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The Pathology Report

16

Andrea Vingiani and Giuseppe Viale

16.1 Introduction

An accurate pathological assessment of core biopsies or resection specimens provides important information on the major features of breast cancer, such as tumor type, size, biological characteristics, lymph node status, stage, and extent of residual disease in case of neoadjuvant chemotherapy, and is crucial for ensuring an appropriate patient management. In the era of molecular medicine and tailored therapies, the pathologic assessment of primary tumor still represents an essential guide for oncologists and surgeons to inform the choice of the best treatment options available for individual patients. Therefore, the management of patients with breast cancer detected through imaging or symptomatic presentation depends heavily on the quality of the pathology service.

Pathologists deal routinely with breast cancer samples, either as surgical resection specimens (both intraoperatively and after fixation and embedding) or as core biopsies and fine needle aspiration cytologies for the preoperative diagnosis of primary tumor or of distant metastases. The foremost means of communication with treating physicians, surgeons, radiologists and radiotherapists (and ultimately the patients) is represented by the pathology report.

Pathology reports may look different in appearance, at the discretion of each specific pathologist, taking into account clinician preferences, institutional policies, and individual practice, but it is mandatory they provide all clinically relevant information. An accurate and detailed pathology report of breast cancer, in addition to the histopathological diagnosis, must include all the prognostic parameters derived from the morphological examination and the immunohistochemical and molecular assessments and the predictive parameters useful for evaluating the efficacy of local and systemic treatments. In this regard, several guidelines for pathological reporting have been issued in the past years, and the most widely used are those of the Association of Directors of Anatomic and Surgical Pathology (ADASP), the College of American Pathologist (CAP), and the Royal College of Pathologists (RCP). These recommendations are drafted as a sort of checklist, a framework to assist pathologist in the completion of an exhaustive pathology report, encouraging healthcare professionals to use common terminology and definitions for breast diseases, and to harmonize the way of classifying breast cancer. In the following sections we will discuss general principles of specimen handling and sampling, as well as all principal parameters to be included in the pathology report, focusing on prognostic and predictive markers.

16.2 Pathology Request Form

An efficient multidisciplinary approach to patient care implies a precise exchange of information among different health-care professionals. Therefore, after the diagnostic procedures or the surgical intervention, any individual specimen submitted to the pathology laboratory should be accompanied by a comprehensive request form, providing the pathologists with all clinically relevant information, inclusive of:

- Patient personal data and demographic information, including name, surname, date of birth, sex, and ethnicity.
- Type of specimen, such as fine needle aspiration cytology; core biopsies; vacuum-assisted biopsy (VAB); lumpectomy and mastectomy, with or without locoregional lymph nodes; and number of specimen containers submitted, identifying each separately.
- Date and time of surgery.
- Clinical history and previous findings, including breast laterality, number and size of lesions, location within the breast, imaging data (mammography, ultrasound, MRI),

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history of previous malignancies, neoadjuvant therapy, including comments on clinical or radiological response. Drawings can be very helpful.
- Previous biopsy or cytology results for each lesion, with relevant details and laboratory of origin, whenever available.
- Method of localization used.
- In resected specimens, drawings or description indicating the position of the orientating clips/sutures. Surgeon should orientate all breast cancer resection specimens. Each breast unit should establish a code of orientation, using either different lengths or number of sutures and/or metal clips or ink. The code should be anatomically relevant and assist in accurate evaluation of the specimen and its margins.
- Whether any relevant marker (most frequently microcalcifications) was identified on imaging of the specimen, if performed.
- For axillary specimens: whether a sentinel lymph node (specifying if an intraoperative assessment is requested or a routine analysis on formalin-fixed, paraffin-embedded sections), a lymph node sampling or a completion axillary dissection (indicating levels dissected) is submitted for pathological examination.

16.3 Specimen Handling and Sampling

Surgical specimens must be handled to ensure good preservation of all the morphological and biological characteristics of the tumor cells. Inadequate fixation may cause extensive morphological artifacts, loss of tissue antigenicity, and degradation of nucleic acids (especially mRNA), making specimen not suitable for a reliable assessment of prognostic and predictive parameters (i.e., hormone receptors, HER2, Ki-67 labeling index, and molecular analysis).

After surgical removal, specimen should be sent immediately to the pathology lab. According to local policies, pathologists may dissect the specimens either on fresh state or after formalin fixation, but in any case a prompt and accurate fixation must be ensured, thus preventing tissue autolysis. In case of delayed sampling, the specimens should be immediately placed in an adequate volume of fixative, at least ten times that of the specimen, and cut through the tumor from the fascial plane toward the surface of the sample, to ensure adequate penetration of fixative into the tumor tissue, especially in large and fatty mammary glands. Refrigeration and vacuum packing may also be helpful in delaying autolysis. The American Society of Clinical Oncology/College of American Pathology (ASCO/CAP) guidelines advocate promptly placing the breast specimens into fixative within 1 h after surgical removal to minimize cold ischemia time and maintaining the samples in 10% neutral buffered formalin (NBF) for 6 h to 72 h, for ensuring best preservation of tissue antigenicity for assessment of HER2 and hormone receptor status [1, 2].

In samples of non-palpable lesions, intraoperative radiography of the specimen or macroscopic examination by a pathologist is particularly useful to confirm the success of the excision procedure. This is also highly recommended for wide local resections (quadrantectomies or lumpectomies), to allow confirmation of the presence of the abnormality and of its location in the specimen, thus facilitating immediate re-excision if the lesion is close to or involving a margin.

Once received in the laboratory, pathologists should examine the specimen, recording the type of excision, its dimensions along the three spatial axes, the presence or absence of skin, and/or nipple and axillary tissue. Relevant surgical margins or the entire specimen surface should be inked so that the margins of excision can be easily determined histologically. Therapeutic surgical procedures, as quadrantectomy or mastectomy, according to Surgical Guidelines for the management of breast cancer, usually require tissue removal from the subcutis to the pectoral fascia, which are considered anatomical planes rather than surgical margins. However, in case of central excision, breast tissue remains at the superficial (close to the nipple-areola complex) surface, requiring careful margin assessment. Therefore, it is important for the pathologist to be aware of the type of excision, in order to manage surgical margins properly.

Afterward, pathologist should slice the specimen at intervals of approximately 3–5 mm, preferably along sagittal planes, enabling easy X-ray mapping of the specimens in case of non-palpable lesions with calcifications or tissue distortion, in order to ensure high-confidence localization. The sampling technique, however, may vary according to the type and size of the samples and also according to local protocols or pathologists' preferences; therefore, some degree of flexibility is allowed. The number of blocks of invasive tumors to be prepared for microscopic examination can vary with tumor size, but it is usually of at least three blocks per tumor nodule. The peritumoral tissue should also be submitted for histology to identify associated DCIS, peritumoral lymphovascular invasion, and to allow surrounding normal breast tissue to be used as an internal immunohistochemical control for the assessment of hormone receptor and HER2 status. It may be possible to sample the lesion and its adjacent radial margin in one block in case of very small lesions, but in the vast majority of the cases, resection margins must be examined in several blocks. Particular attention must be paid to the areolar margin, due to high gland density and possible tumor extension

in lactiferous ducts, particularly for DCIS. In mastectomy specimens, sagittal sections of the nipple should be taken to exclude Paget's disease, while a coronal section of nipple and retro-areolar tissue is recommended to assess possible nipple duct involvement by DCIS.

For DCIS specimens, the number of blocks sampled is variable according to the size of the specimen and of the lesion. For small specimens, especially when radiologic assessment is unavailable, sampling of the entire tissue is recommended. For larger specimens, the pathologist should sample representative blocks (at least one block for each centimeter of the lesion) from the entire involved area, to scrutinize the sample for any possible area of invasive carcinoma, and including the site of any previous core biopsy.

Ultimately, details of the macroscopic features of the specimen must be recorded, especially tumor size and distances to all margins. In the presence of multiple tumors, the distance between tumors themselves and between each tumor and resection margins should be recorded. It is recommended to sample the tissue between tumor nodules, to ascertain if the neoplastic foci are truly separated (multifocal or multicentric tumors) or instead interconnected. The axillary tail of the specimen should be inspected for the presence of intramammary or low axillary lymph nodes.

Neoadjuvant systemic therapy is frequently administered to patients with large, locally advanced, or inflammatory breast cancers, with the aim to reduce the tumor size allowing breast-conserving surgery and tumor downstaging. It also provides the opportunity to assess response to treatment after a reasonably short time of exposure to the treatment (see related chapter). However, significant difficulties and variability exists in methods for pathologic assessment of response to neoadjuvant therapy. Recently guidelines issued by the Breast International Group-North American Breast Cancer Group (BIG/NABCG) [3, 4] recommend practical methods for a standardized pathologic assessment of the breast specimen following neoadjuvant therapy. Briefly, it is mandatory to identify macroscopically the tumor bed before any sampling and to record the two axes of the largest cross section of the entire area involved. Obvious remaining tumor should also be measured. It is strongly recommended that an image of the sliced specimen be taken (photograph or drawing) and then used to create a map of the carefully oriented tissue blocks collected. This will allow pathologists to obtain an accurate and comprehensive histological image of residual tumor, ultimately assuring a precise assessment of residual disease and staging. Extent of sampling should be determined by the pretreatment tumor size; an entire cross section of the tumor bed taken for each cm of the pretreatment tumor size (for a total number of approximately 15 blocks in most cases) should be sufficient to reliably document the pathological response.

16.4 The Pathology Report: A Synopsis

The following are the main parameters that should be carefully evaluated and clearly reported in the pathology report. Their assessment methods and clinical relevance will be briefly discussed.

16.5 Tumor Type

Breast cancer is considered a heterogeneous disease, made up of several different subtypes with variable morphological and biological features, different prognosis and response to systemic therapy. WHO histopathologic classification is based on characteristics seen upon light microscopy of biopsy specimens [5]. Two most common histopathological types collectively represent approximately 70–80% of breast cancers, namely, invasive ductal carcinoma, no special type (IDC NST) or invasive lobular carcinoma (ILC).

Among less common tumor histotypes, some "special" tumor types are per se associated with intrinsically peculiar prognostic profile. Tubular and cribriform carcinomas, for example, are characterized by an almost indolent clinical course with an extremely good overall survival [6], and the adenoid cystic carcinomas carry a very favorable prognosis in the vast majority of the cases [7]. On the contrary, metaplastic carcinomas are associated with significant worse clinical outcome then the IDC NST [8].

The fact remains, however, that for the vast majority of breast cancer (IDC NST and ILC), morphological classification is unable to meaningfully reflect the vast heterogeneity in terms of biological features, prognosis and response to systemic therapy, failing to assist the oncologist in planning adequate systemic treatment. It is also arguable if IDC and ILC do actually reflect clinical differences, and whether ILC per se constitutes a prognostically favorable group [9, 10].

16.6 Tumor Size

An accurate measurement of tumor size is mandatory, as it represents the first parameter of breast cancer staging. TNM classification still represents one of the most powerful prognosticators in breast cancer [11], being statistically correlated with risk of recurrence, metastatization, and overall survival. Identification of the tumor edge is also a prerequisite for a reliable assessment of resection margin status.

The maximum dimension of invasive tumors should be measured macroscopically, paying attention to irregularly shaped or multi-lobulated lesions. When tumor measurement is not feasible, then the tumor size identified by imaging, based on ultrasound, mammography, or MRI, should be used

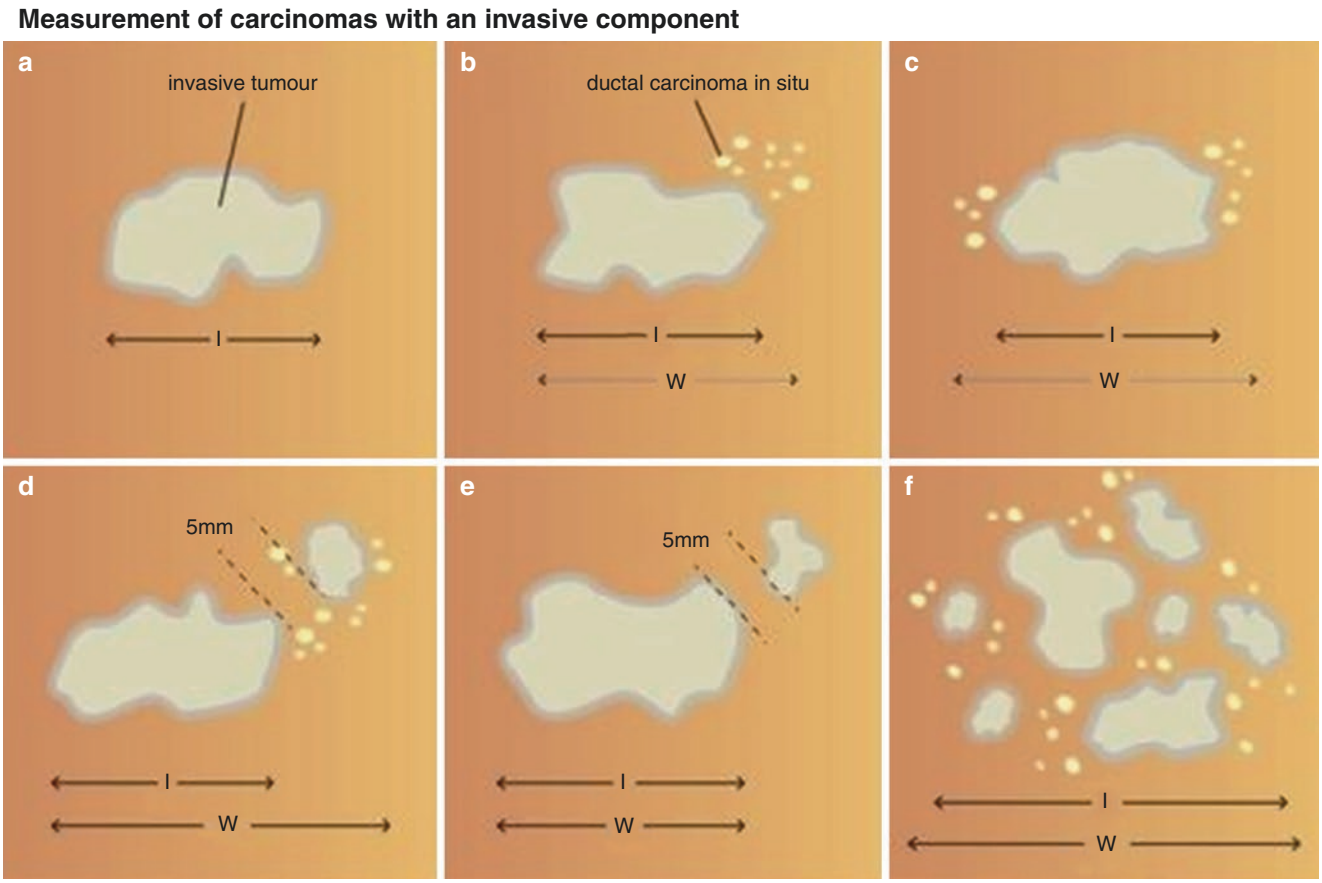

Fig. 16.1 Illustrations of how to measure invasive and whole tumor sizes in various scenarios [12]

as the best available record of true tumor size, replacing pathological size assessment. In case of discrepancy between the macroscopic and the microscopic size occurs, then the latter should be recorded, provided that the plane of the maximum dimension of the lesion has been included in the slide. The assessment of the whole tumor size including in situ carcinoma should be recorded, reporting also relative percentages of invasive tumor and DCIS. In tumors composed predominantly of DCIS but with multiple foci of (micro)invasion, measurement of the invasive tumor should correspond to maximum axis of the area occupied by invasive foci, as shown in Fig. 16.1, along with other frequent scenarios.

On rare occasions, pathologists may find it challenging to determine whether two adjacent tumor foci represent satellite foci or one lesion mimicking this process due to plane of sectioning. In this regard, the presence of intervening normal tissue and increasing distance between foci are features suggesting that these are more likely to be multiple foci than a single process. A distance of 5 mm or greater is often used to define separate foci. In case of clear-cut distinct multiple tumor masses, pathologists should record if the neoplastic foci are in the same breast quadrants or at a distance of less than 5 cm (multifocal tumors) or in different quadrants or at a greater distance (multicentric tumors).

16.7 Histological Grade

Invasive carcinomas are routinely graded, and grade is now widely recognized as a powerful prognostic factor, significantly associated with clinical outcome [13–15]. Assessment of histological grade has become more objective with modifications of the Patey and Scarff [16] method first by Bloom and Richardson [17] and more recently by Elston and Ellis [18]. Histological grading involves the assessment of three components of tumor morphology: tubule/acinar formation, nuclear atypia/pleomorphism, and number of mitoses. Each parameter is scored from 1 to 3, and the sum gives the overall histological grade as follows: Grade 1 (well differentiated) = scores of 3 to 5, Grade 2 (moderately differentiated) = scores of 6 to 7,

and Grade 3 (poorly differentiated) = scores of 8 or 9. Below are briefly discussed criteria involved in tumor grading:

- *Tubule/acinar formation*: all tumor area should be scanned, assessing semiquantitatively the proportion occupied by tubule formation. This assessment is generally carried out during the initial low-power scan of the tumor sections. Tumors showing >75%, 10–75%, or <10% of tubule formation are scored 1, 2, or 3, respectively.
- *Nuclear atypia/pleomorphism*: assessed comparing tumor nuclear size and shape with normal luminal cells. This is the parameter mostly affected by interobserver variability; breast specialist pathologists seem to report higher grades than nonspecialists [19].
- *Mitoses*: accurate mitotic count requires optimally fixed and processed specimens. Mitoses should be counted in ten high-power fields (40× objective). The mitotic score is dependent
- on the high-power field diameter; in this regard tables of conversion with different scoring tiers according to the actual field diameter of the microscope are available. At least ten fields should be counted at the periphery of the tumor, where it has been demonstrated that proliferative activity is greatest [20, 21]. If there is variation in the number of mitoses in different areas of the tumor, the area with the highest mitotic count should be taken into account. If the mitotic score falls very close to a score cut point, additional ten high-power fields should be evaluated, assigning the highest score.

In core biopsies, notwithstanding paucity common low cellularity of the samples, assessment of grade is recommended, especially if the patient is a candidate to neoadjuvant treatment. There is about 70% agreement of grade on core biopsy with the corresponding surgical specimen [22, 23]. If both core biopsy and surgical specimen are available, grading should be scored on the latter. Assessment of grade in the surgical specimens after neoadjuvant systemic therapy may be unreliable, due to the effect of the cytotoxic drugs on the morphology and the mitotic index of the tumor cells.

16.8 Peritumoral Lymphovascular Invasion

Lymphovascular invasion (LVI) mirrors the ability of cancer cells to invade lymphatics and blood vessels, and it is correlated to a higher likelihood of nodal or distant metastases. LVI in a peritumoral location is unanimously regarded as an important prognostic factor in patients with lymph node-negative invasive breast cancer, providing independent information about both local recurrence and survival [24–26]. It is therefore important to record in the pathology report whether or not it is present. Given the difficulties in the morphological distinction between lymphatics and blood vessels, findings should be categorized as "lymphovascular spaces" rather than as specific channels. This is supported by evidence identifying that most tumor emboli are present in lymphatic channels.

At the microscopic level, stromal retraction artifact around neoplastic cell nests can mimic vascular invasion; therefore, a clear rim of endothelium should be identified. Other clues in recognizing lymphovascular invasion is the presence of nearby vascular channels or the location of tumor cells within spaces with erythrocytes and/or thrombi

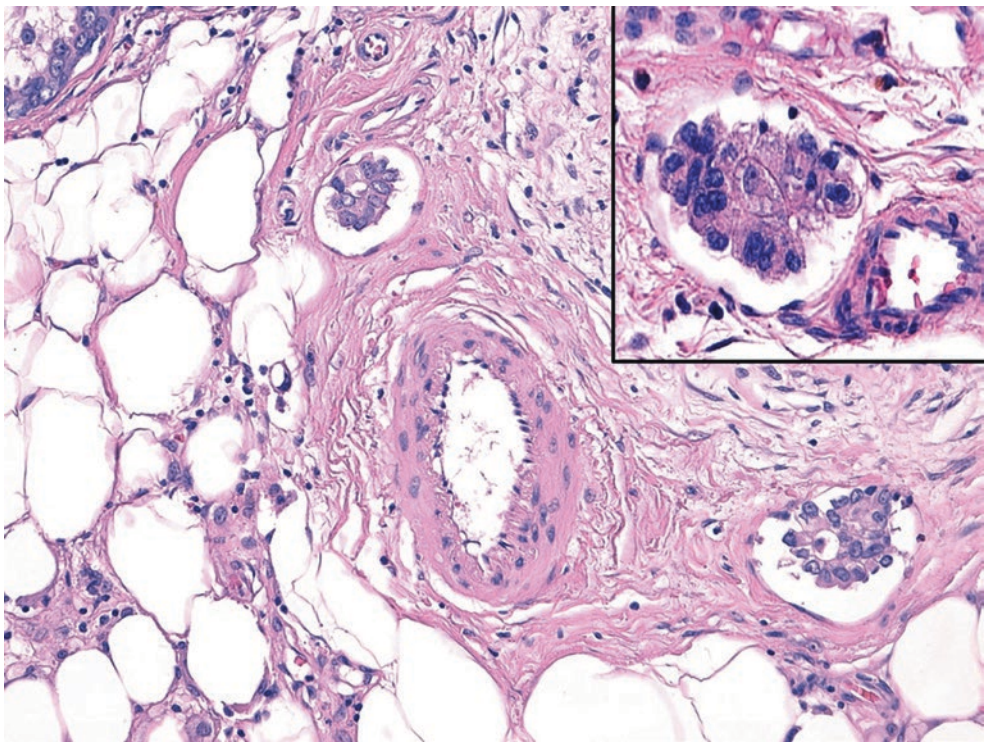

Fig. 16.2 An example of lymphovascular invasion

(Fig. 16.2). In difficult cases immunohistochemistry can be of help to identify the endothelial lining (CD34, CD31, and D2-40 antibodies) [27].

16.9 Surgical Margins Status

Breast-conserving surgery (BCS) in combination with adjuvant local and systemic therapies has become the standard of care for early small tumors, thus reducing physical and psychological morbidity. Despite these advantages, BCS has a higher risk of local recurrence than mastectomy [28–33]. The strongest predictor of local recurrence is surgical margin status [34–36].

According to the number of positive margins and the remaining amount of breast tissue, positive margins are managed with re-excision or mastectomy, eventually resulting in poor cosmetic outcome and high medical cost. Since early invasive cancer and DCIS nowadays represent a significant fraction of breast surgical specimens, the pathologic assessment of surgical margins is crucial, requiring close correlation between the surgical procedure and pathological examination. In particular, pathologists should be aware of the depth of tissue excised and whether the surgeon has excised all the tissue from the subcutaneous to the pectoral fascia. All distances between invasive cancer and DCIS should be recorded or, at least, the closest ones. According to current recommendations, margins are considered free of invasive tumor when the ink does not touch the tumor. For DCIS, margins are considered free when the closest tumor nest is 2 mm away from the surgical margin.

As previously mentioned, careful orientation of the surgical specimen is mandatory, as this prevents discordance with postoperative margin orientation, which occurs in 31% of surgeries [37]. In comparison to permanent histopathologic staining, intraoperatory pathological assessment of surgical margins may result in significant decrease of re-excisions. In particular, macroscopic examination, frozen section analysis, and imprint cytology have been demonstrated to be associated with re-excision rate of 3–11%, against an average 35% rate for permanent histopathologic staining-surgical margin assessment [38, 39].

16.10 Nodal Status and Sentinel Lymph Node Biopsy

Axillary node status is the most important prognostic indicator in breast cancer. Therefore, careful assessment of nodal status is mandatory. If axillary dissection has been performed, all lymph nodes must be carefully dissected and examined histologically. Pathology report should include the total number of lymph nodes identified and the number of involved lymph nodes, specifying whether macro- or micrometastases. Of note, nodes with isolated tumor cell only (<0.2 mm) are not considered positive for metastasis [40, 41].

However, axillary dissection is not infrequently associated with side effects, such as arm lymphedema, paresthesia, pain, and motor deficit. Moreover, with the implementation of screening programs, an increasing number of patients with node-negative early breast cancer were subjected to unnecessary axillary dissection. Hence, the need for a diagnostic procedure capable of discriminating patients for whom completion axillary dissection could be avoided. Sentinel lymph node biopsy (SLNB), a technique initially devised for penile cancer and melanoma, relies on the assumption that lymphatic spread of cancer cells occurs orderly and sequentially along the lymphatic drainage; hence, there must be a lymph node supposed to be the first metastasis recipient and from which the disease can subsequently spread to the remaining lymph nodes. Phase III clinical trials by Veronesi's and Giuliano's groups convincingly demonstrated that breast cancer patients with a negative SLNB could safely avoid axillary dissection [42–44]. Since these seminal works, SLNB entered the clinical arena, and pathologists put their efforts in defining the most accurate way for SLNB analysis, leading to a surprisingly variegated panorama, with no universally accepted protocols. Some institutions adopted an intraoperative frozen section assessment, to avoid a second surgery in case of a positive SLNB, using different protocols with regard to the number of sections examined and the cutting intervals; others adopted assessment of lymph node status on permanent sections, also using immunohistochemical stains, with the goal of achieving high sensitivity. More recently, intraoperative molecular assessment, using reverse transcription-PCR assays for cytokeratin-19 (one-step nucleic acid amplification, OSNA), entered the clinical practice [45–47]. The obsession to look for even minimal sentinel lymph node involvement, however, was eventually challenged by the evidence that patients with isolated tumor cells or micrometastasis only in the sentinel lymph node could be safely spared completion axillary dissection without any adverse effect on outcome [48]. This led to questioning the need for axillary dissection also for patients with small and clinically node-negative breast cancer, but histologically positive sentinel node. The ACOSOG Z0011 trial randomized 891 patients with clinically T1–2, breast cancer, and histologically positive SLNB, to undergo axillary dissection or not. When the ACOSOG Z0011 was initially reported with a median follow-up of 6.3 years, regional recurrence after SLND alone for women with 1 or 2 positive sentinel lymph nodes was surprisingly low (0.9%), and completion axillary dissection did not significantly reduce regional recurrence or improve survival [49, 50].

Further data analysis, with nearly 10 years of median follow-up, still showed a remarkably low regional recurrence rate of 1.5% for SLND alone [51].

Following the report of these results, completion axillary dissection is no longer recommended for patients with small early breast cancer undergoing breast-conserving surgery and whole breast irradiation, even in case of metastasis to one or two sentinel lymph nodes.

16.11 Biological Features of Breast Carcinoma

Adjuvant systemic therapy of breast cancer is mainly informed by the biological characteristics of the primary tumor, including hormone receptor and HER2 status, and the assessment of the proliferation fraction [52]. It is therefore mandatory that the final pathological report includes an accurate evaluation of these parameters.

As previously mentioned, a reliable assessment of these biological features requires an optimized pre-analytical phase, with proper fixation of the specimen. One of the most important steps for optimal testing is the choice of the block to be submitted for the assays: it should be taken at the invasive edge of the lesion, including normal breast parenchyma, and must be representative of the invasive component of the tumor. In bilateral breast cancer, samples from both tumors should undergo biological characterization, given the high frequency of phenotype discordances in bilateral cancer; for multifocal or multicentric disease, ideally all the different foci should be evaluated, but in the vast majority of cases they exhibit similar morphological and biological phenotype. A reasonable approach would be to assess first whether the different tumor foci show the same morphological features (i.e., tumor type and grade) or they are different. In the former case, it may be acceptable to assess hormone receptors, HER2 and proliferative index in only one nodule, whereas in the latter it is recommended to test all the foci that are morphologically different. In multifocal or multicentric disease, in case the nodule assessed for biological characteristic shows a triple negative phenotype, it is highly recommended to test further foci, seeking for clones with different biological features amenable to hormonal or targeted therapy.

16.12 Estrogen and Progesterone Receptor Status

ER plays crucial roles in breast carcinogenesis; it was first identified in the 1960s and used in breast cancer clinical management since mid-1970s. It is universally considered one of the most important biomarkers for breast cancer classification, as a primary indicator of endocrine responsiveness, thus guiding oncologist in planning patient treatment [53]. ER status has been shown to be the major determinant of breast cancer molecular subtype by gene expression profiling studies [54]. ER-positive tumors comprise up to 75% of all breast cancer patients [55] and are largely well differentiated, less aggressive, and associated with better outcome after surgery than the ER-negative ones [56, 57]. ER has been considered as the most powerful single predictive factor identified in breast cancer [58–60], given the fact that approximately 50% of patients with ER-positive disease benefit from endocrine therapy [61].

The panelists of the St. Gallen Consensus in 2009 suggested to consider positive for ER and progesterone receptor (PgR) those tumors showing at least 1% immunoreactive cells [62]. This definition has been subsequently endorsed by the ASCO/CAP guideline recommendations for ER and PgR immunohistochemical testing [63]. Reporting the actual percentage of neoplastic cells showing definite nuclear immunoreactivity has also been recommended, because the higher the number of positive cells the larger is the expected benefit from endocrine therapies. Scoring system taking into account also the staining intensity (like the H-score or the Allred score) is considered optional. The ASCO/CAP guidelines covered technical aspects of the pre-analytical and analytical steps of the immunohistochemical, interpretation, scoring, and reporting of the results, aiming to increase accuracy and reproducibility. One of the most useful recommendations to avoid false-negative results in ER testing is to evaluate systematically the immunoreactivity of the nonneoplastic breast tissue surrounding the tumor. Ductal and lobular luminal cells are invariably heterogeneous for ER and PgR immunoreactivity, whereas myoepithelial and stromal cells are invariably negative thus providing a built-in positive and negative control of the sensitivity and the specificity of the reaction (Fig. 16.3).

While in certain subsets of cases ER-positive tumors may be negative for PgR, conferring lower sensitivity to antiestrogen therapy, especially in the metastatic setting, the reversed phenotype (ER negative and PgR positive) is very rarely true. Almost all the cases with such an aberrant phenotype are due to a false-negative assay for ER or, less frequently, a false-positive assay for PgR, and the pathologists should be encouraged to repeat the test, possibly on a different block before rendering this unusual report.

16.13 HER2

Human epidermal growth factor receptor 2 (HER2)-positive [1] breast cancer (BC) accounts for 15–20% of early breast cancer, and it is characterized by an aggressive behavior and poor response to conventional chemotherapy (CHT) [64, 65]. HER2 drives tumorigenesis mostly through protein overexpression in his wild-type form and pathway hyperactivation. Cancer promotion by HER2 kinase domain activating

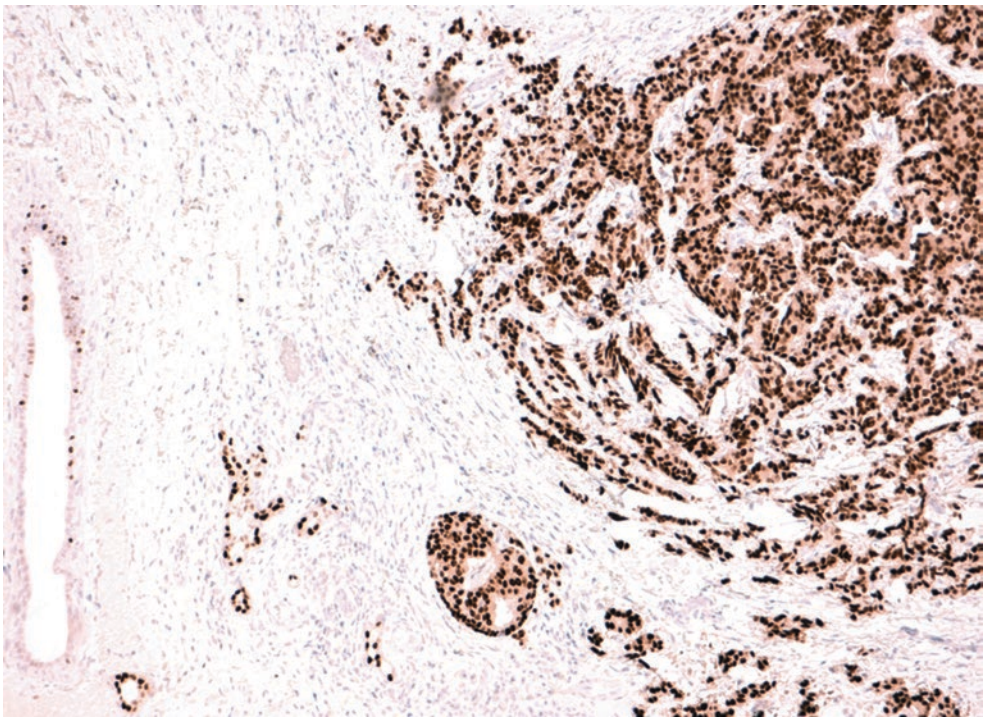

Fig. 16.3 Invasive ductal carcinoma NST showing diffuse (100%) intense positivity for ER. On the left a normal breast duct, showing heterogeneous staining

mutations has been rarely (3%) reported in the absence of protein overexpression [66–68]. The development and the clinical use of HER2-targeted therapies (antibodies and tyrosine kinase inhibitors) [69] led to a dramatic improvement of the outcome for patients with HER2-positive (HER2+) breast cancer [70–75]. HER2 pathways may be even more efficiently inhibited by combination therapies (dual blockade), as demonstrated in the metastatic and neoadjuvant setting [76–79], and currently tested within phase III randomized trials in the adjuvant setting [80]. Despite the efforts for standardizing HER2 testing, its reproducibility still represent a significant issue: central pathology review of locally assessed samples collected within prospective clinical trials reported concordance rates in the assessment of HER2 status by immunohistochemistry or in situ hybridization assays ranging from 77.5 to 96% [81–83]. In this regard, guidelines describing how to optimally perform the immunohistochemical (IHC) and in situ hybridization (ISH) assays for assessing HER2 status and evaluate and score the results have been issued and regularly updated [1]. Briefly, the 2013 ASCO/CAP guidelines define HER2-positive (score 3+) breast carcinoma as tumors containing more than 10% of cells with complete and intense circumferential membrane staining by IHC. ISH-positive breast carcinoma is defined as showing an average HER2 copy number ≥6.0 signals/cell or average HER2 copy number ≥4.0 signals/cell and a HER2 to chromosome 17 centromere (CEP17) ratio ≥2.0. Cases presenting weak to moderate circumferential membrane staining in more than 10% of tumor cells, or intense, complete and circumferential membrane staining in less than 10% of tumor cells should be classified as equivocal (score 2+) by IHC, while cases presenting HER2 to 17 centromere (CEP17) ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals per cell are considered equivocal by ISH. Equivocal cases require further assessment with the alternative assay or re-testing with the same assay of different tumor blocks or synchronous nodal metastases if available. Incomplete, faint, or barely perceptible membrane staining in more than 10% of tumor cells (score 1+) and no staining observed or incomplete, faint, or barely perceptible membrane staining in less than 10% of tumor cells (score 0) would confidently classify breast cancers as HER2 negative by IHC, while ISH-negative cases are characterized by a HER2 to chromosome 17 centromere (CEP17) ratio <2.0 with an average HER2 copy number <4.0 (Fig. 16.4).

16.14 Ki-67 Labeling Index

Tumor proliferation is one of the most powerful tools in breast cancer prognostication. The protein identified by the Ki-67 antibody is expressed in all proliferating cells during late G1, S, G2, and M phases of the cell cycle, peaking in the G2-M phases. In clinical practice, tumor proliferative fraction is most commonly assessed by the immunohistochemical staining of the Ki-67 antigen, using the MIB-1 monoclonal antibody [84].

The prognostic and predictive value of tumor proliferation has been extensively investigated in both the neoadjuvant and adjuvant settings [85, 86] and has been corroborated by gene expression analysis and molecular prognostic signatures, whereby the identification of intrinsic breast cancer molecular subtypes (i.e., Luminal A vs. Luminal B) or the distinction between aggressive or more indolent tumors

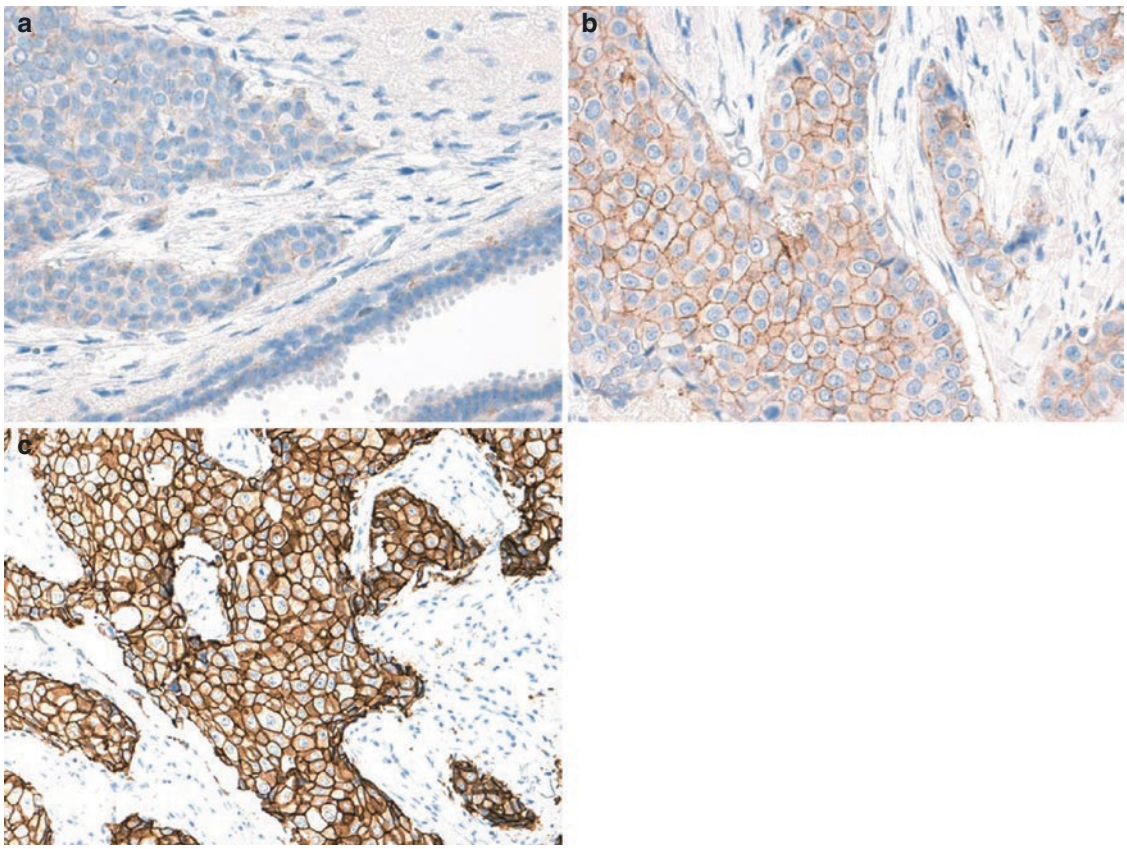

Fig. 16.4 Immunohistochemistry for HER2. Score 1+ (**a**); score 2+ (**b**); score 3+ (**c**)

relies mainly on proliferation-related genes [54, 87–92]. Therefore, accuracy of Ki-67 scoring is still a crucial issue, and huge efforts had been spent in improving consistency and in identifying the ideal cutoff value.

In 2010, the panelists of the International Ki-67 in Breast Cancer Working Group met in London and issued comprehensive recommendations aiming to achieve a harmonized, reproducible, and accurate methodology. Substantially, they suggested to assess Ki-67 labeling index on full-face sections, in at least three high-power (×40 objective) fields after an initial overview of the whole section, scoring at least 500 malignant invasive cells, preferably at the invasive edge of the tumor [93].

In their pivotal study, Cheang and colleagues [94] showed that a 14% cutoff was reliably able to discriminate tumors belonging to the Luminal A and Luminal B molecular subtypes. They found a Luminal A prevalence among ER-positive samples of approximately 60%. On the contrary, applying this same cutoff, other authors found an opposite prevalence of Luminal A and Luminal B cases. Consequently, at the 2013 St. Gallen Conference [95], the 14% cutoff for Ki-67 was challenged, and the majority of the panelists proposed to raise it to 20% for a better subclassification of luminal tumors. At the same time, Prat and colleagues [96] suggested to include high PgR expression (>20%) as an additional parameter for identifying the Luminal A subtype, along with ER positivity, HER2 negativity, and <14% Ki-67 labeling index. Maisonneuve et al. tested these new parameters in 9415 ER-positive and HER2-negative early breast cancer patients, treated between 1994 and 2006 and followed up at the European Institute of Oncology in Milan [97]. According to the 2011 St. Gallen criteria (Ki-67 cutoff of 14%), they found that 33% of the tumors would have been classified as Luminal A and 66% as Luminal B. Using the 2013 criteria (Ki-67 at 20% and adding PgR with the 20% cutoff), 43% of the tumors qualified for Luminal A and 57% for Luminal B. Interestingly, distant disease-free interval of the patients with low-proliferating tumors (Ki-67 < 14%) was not affected by PgR. Conversely, patients with tumors showing an intermediate Ki-67 labeling index (between 14 and 19%) had a significant different outcome according to PgR status, suggesting to classify as Luminal A tumors with either low (<14%) Ki-67 labeling index or with an intermediate labeling index (14–19%) and PgR of >20%. Luminal B tumors would be defined by either high Ki-67 labeling index (20% or more) or an intermediate Ki-67 and PgR _20%. Using this definition, 52% of the 9415 tumors qualified for Luminal A and 48% for Luminal B, with a significantly different clinical outcome of the patients (HR: 1.75, 95% CI: 1.42–2.11), after adjustment for clinicopathological variables including pT, pN, tumor grade, peritumoral vascular invasion, menopausal status, and systemic therapy.

16.15 Epilogue

The histopathological and biological characteristics of breast carcinoma are essential parameters to inform the choice of the systemic treatments. Hence, the pathology report of breast cancer must be complete and accurate, and include all the relevant features of the tumor. Recommendations have been issued by several national and international organizations on how to best evaluate and report these features and are continuously updated. It is the responsibility of each individual pathologist to follow all the available recommendations and guidelines strictly. The role of the pathologists in the multidisciplinary approach to breast cancer patients cannot be overemphasized, and the pathology report is their most important contribution.

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Part IV

Imaging

Radiological Screening of Breast Cancer: Evolution

17

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Abbreviations

AWBU	Automated whole-breast ultrasound
BC	Breast cancer
CAD	Computer-aided diagnosis
CC	Case-control
CE	MRI Contrast-enhanced magnetic resonance imaging
CI	Confidence interval
CNBSS	Canadian National Breast Screening Study
CRT	Chest radiation therapy
DBT	Digital breast tomosynthesis
HIP	Health Insurance Plan
HR	High risk
IBM	Incidence-based mortality
IT	Information technology
LTR	Lifetime risk
MR	Magnetic resonance
MS	Mammography screening
NBCSP	Norwegian Breast Cancer Screening Programme
NCR	Nordic Cochrane review
NNS	Number needed to screen
OR	Odds ratio
PPV	Positive predictive value
RCT	Randomized controlled trial
RR	Relative risk
STC	Swedish Two County
TNBC	Triple negatibe breast cancer
US	Ultrasound
USPSTF	US Preventive Services Task Force

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17.1 Normal Risk Population

Alfonso Frigerio

Abstract Mammography screening is one of the revolutionary advances in the fight against breast cancer, alongside breast-conserving surgery. Few medical interventions have been so extensively evidence-based and yet subjected to persistent critiques. The clear scientific evidence of the efficacy of screening in reducing breast cancer mortality is discussed. Benefits provided by screening are substantial, well above any negative effect. In the age of modern treatment, early detection still contributes to breast cancer mortality reduction.

A full appreciation is advocated for organized screening programs and the added value they provide in terms of high quality, equitable health service, and as the optimal environment where best capitalize on the new advances in treatment. Future evolution might include (a) tailored, risk-based protocols, in the first place extending the age range of offered screening; (b) new imaging tools; and (c) optimization of existing programs, through better monitoring, training, and research—always abiding by the big caveats: evidence of efficacy, incremental cost-effectiveness, and sustainability. Both screening and treatment have merits in achieving mortality reduction. It would be clever to recognize their mutual enhancing power and devote resources to a very appropriate topic for research: how early detection might or should change the treatment of breast cancer.

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17.1.1 Introduction

Breast cancer (BC) has been a curse for women's health since historical records exist, back to the ancient civilizations thousands of years ago. Our generation has had the privilege to witness the first real breakthrough in a long-standing story of sufferance and defeats. It was only the final decades of the twentieth century that brought about decisive innovations, in both diagnosis and treatment of this ominous disease. New medical therapies were introduced, and radiation oncology was developed, both attaining a relevant role in treatment protocols, especially so in their adjuvant capacities.

However, the two major advances came (a) with the introduction of breast-conserving surgery and (b) with the prospective, randomized controlled trials (RCT) that demonstrated for the first time in history the possibility to reduce BC mortality through early diagnosis, by the systematic application of mammography screening (MS).

The therapeutic equivalence of quadrantectomy to mastectomy in the treatment of small cancers, originally suggested and then scientifically demonstrated by Veronesi and others [1, 2], presented women with an amazing chance to avoid the traditional, mutilating, standard treatment of the last century, namely, Halsted's radical mastectomy.

Almost at the same time, population-based radiological (mammographic) screening was proposed and validated as a major health achievement that made it possible to decrease BC mortality by treating the disease when it was still localized in the breast.

Indeed, these two major innovations enhanced each other's benefits, as early mammographic diagnosis provided surgeons with more and more small cancers, which could be a candidate for the new breast-sparing surgery. Early detection allowed also for the adjuvant therapies, both medical and radiation-based, to achieve extraordinary results in disease control. Through this mutual support, early diagnosis in conjunction with more effective treatment opened the way to a new era in the fight against BC.

It is ironic that in recent years, it was just this enhancing, synergic action that offered one of a series of spurious arguments to discount the value of early detection as a powerful measure to control BC mortality, in this epoch of developing new therapeutic regimens. Such argument has given support to a great deal of data misinterpretation and a long sequence of futile controversies.

The present pages shall try to summarize and highlight the clear, overwhelming scientific evidence on the efficacy of MS in reducing BC mortality and the importance of building and keeping up large population-based screening programs as a needful strategy in order to best capitalize all the treatment advances that have been and are being developed.

It will be shown how current estimates of benefits achievable through MS are substantially undervalued, and it will also be suggested that the future evolution of BC management should strive to include an innovative rethinking of some concepts that form the basis of pathological representation, description, and classification of breast diseases, taking into consideration many new pieces of knowledge derived from the screening experience. This new perspective could bring about a change in the fundamental concepts of BC treatment, at least when the tiny, screen-detected cancers are involved. New tailored treatment protocols, based on a full appreciation of different parameters of tumor characterization, should be developed. These in turn would make it negligible the concern that has been raised on the overdiagnosis at screening (and the ensuing overtreatment) of a proportion of indolent cancer cases.

In the near future, alongside some anticipated technology-based modifications of the protocols (the subject of subsequent chapters in this book), the evolution of MS will have to consider many different ways of customizing the screening intervention, according to various risk factors, in order to maximize the cost-effectiveness of the system.

17.1.2 The Evidence

Few medical procedures and interventions have been so extensively studied, proven effective, thoroughly evidence-based as MS, and yet discussed and subjected to persistent critiques and unrelenting, often specious attacks.

Since the pioneering New York Health Insurance Plan (HIP) project [3], a wealth of studies, trials, and service programs formed the basis for hundreds of publications that have been dedicated to MS, so that an exhaustive bibliography is practically impossible to collect and report. It is worthy of note and almost a paradox that the prospective, randomized controlled trials (RCTs) where we base the core of our knowledge have been subjected to far more analyses and meta-analyses than the original number of trials. Therefore, references at the end of this chapter should be considered as a very selective choice of relevant contributions. A comprehensive list of references (up to year 2012), as well as a very knowledgeable analysis of their contents, may be found in the special supplement issue of the *Journal of Medical Screening* edited by Paci and reporting the efforts of the Euroscreen Working Group in providing in-depth, expert discussion of the literature on MS, as well as precious, recent data from many European countries [4]. It is convenient to remark at this point that from the immense database accumulated through the screening experience, the best researchers have been able to draw illuminating concepts on the natural history of BC [5].

It was just this incredible number of publications, combined with the substantially variable quality among them and with the extreme complexity of the subject matter, that in the first place made it possible and then immensely contributed

to the diffusion of a still unending trail of largely futile controversies. However, a portion of the conflicting views on MS may in fact derive from different ways of expressing the same results, rather than from substantial disagreement on the data available.

It is still unfortunate that what has been opportunely defined as "an active anti-screening campaign […] based on erroneous interpretation of data from cancer registries and peer-reviewed articles" [6] has been kept alive over the last two decades to this day, with a disconcerting pattern of following waves. This process may be described as a "provocative sequence" of:

(a1) Main question
(a2) Scientific proof provided
(a3) Evidence questioned on poor or unsubstantiated terms
(a4) Evidence (to some extent) conceded, but then
(b1) New question set forward
(b2) Scientific proof provided - etc, through (d4)

According to this pattern, the subsequent questions and critical waves against MS can be summarized as follows (a discussion of these points and relevant references are given below):

(a) Can MS reduce BC mortality?—**the efficacy issue**: (a1) evidence provided by the big RCTs; (a2) evidence questioned, most pugnaciously by the Nordic Cochrane Centre; (a3) evidence eventually (to some extent) conceded in subsequent articles; and (a4) new issue set forward about effective reproducibility of trial results into public health practice.

(b) Can MS service programs reproduce the results of the RCTs and actually save lives in a sustainable way in the context of the health-care system?—**the effectiveness issue**: (b1) evidence provided by a large number of observational studies; (b2) evidence questioned, mostly on the basis of methodologically poor "ecological" studies, lacking information about actual exposure of women to MS; (b3) evidence eventually conceded in subsequent articles; and (b4) new issue set forward about "harms" of screening surpassing the possible benefits.

(c) Are the benefits provided by MS more substantial than any unwanted effect that it may produce?—**the harm/benefit balance** analysis of MS: (c1) evidence of a favorable balance provided by many researchers and prominently in the Euroscreen Working Group analysis; (c2) evidence questioned, especially on the basis of grossly inflated estimates of overdiagnosis; (c3) evidence conceded, most authoratively by the UK Independent Panel [7], the "Marmot report;" (c4) new issue set forward about any remaining significance of the role of early detection in the new age of effective cancer treatment.

(d) Even after MS was proved valid and effective by RCTs and even conceding that its side effects could be minor in respect to the potential benefits, does early detection through MS still hold its meaning in the new era where very effective new treatments for BC have become available? Is it not the case that most of the BC mortality reduction that has been recently observed should be credited to treatment rather than MS?—**the "expired validity" issue** of MS: (d1) evidence has been provided confirming a substantial net benefit of screening on top of the achievements of treatment and (d2) discussion on this point (d3–d4) will be commented in the following pages.

It might reasonably be argued that the above sequence respects the very basics of scientific debate. This would be certainly true, if such sequence was not undermined, as in this case, by an almost breathtaking, unrelenting introduction of methodologically weak or clearly erroneous arguments.

Then, for all these questions, is there any real room for genuine controversy?

The clear, plain answer has to be no.

It is soundly proved that MS substantially does reduce BC mortality and is effective in actual health-care practice; the benefits produced by MS are large and substantial, well above any negative effect.

MS does still substantially contribute to BC mortality reduction even in the age of modern treatment.

The exception where there is indeed space for further analysis is overdiagnosis which, although well compensated for by the mortality reduction benefit, is an extremely complex topic that deserves a more thorough discussion.

In the above series, one more argument has been purposely skipped that had at one point been raised to fuel the debate, namely, the lack of evidence about MS reducing general (all-cause) mortality in the population. This appears the most specious in a series of largely specious arguments. As clearly stated in the Marmot report, reducing BC deaths by 20% in ages 55–79 years would yield a 1.2% reduction in all-cause deaths. The RCTs were not designed for and "are not of sufficient size to allow such small reductions to be reliably estimated. Hence, a statistically non-significant effect for all-cancer or all-cause deaths in the trials cannot be interpreted as evidence against a reduction in BC deaths" [7].

Rather, two key points deserve to be highlighted already at this point, as central in the debate:

1. The quality evaluation of the studies considered has to be factual and circumstantial, i.e., their internal validity must be convincingly proven.
2. The importance of very long follow-up times. These are imperative as we aim at the precise estimate of the benefits involved with the early detection of a group of diseases like BC, which are characterized by a variable, often very long natural history.

Of the above sequence, **issue (a)** will be discussed at this point.

Issues (b–d) will be the subject of paragraph three (service screening).

17.1.2.1 The Efficacy Issue

MS involves an active intervention on large populations over extended times, i.e., huge numbers of study subjects, observed for very long study and follow-up periods, with many parameters to consider, subjected to a number of possible biases. The rationale for screening is advancing the time of diagnosis in order to improve prognosis through earlier treatment. Thus, the apparent incidence of BC has to increase at the start of the process. Also average time from diagnosis to death will increase, introducing a powerful bias (lead-time bias). This might induce erroneous estimates of benefits and harms of MS, when not judiciously taken into account. However, MS efficacy can be stated with great confidence, thanks to available scientific data of the best quality in order to overcome lead-time bias, as mortality data from RCTs are available to support it.

The wealth of evidence provided by a number of excellent, more recent observational studies will also be considered and highlighted in the next paragraph.

The story itself of MS was in fact born with a randomized study. It originated from a brilliant idea back in the 1950s–1960s, when the new technical tool of mammography was suggested [8] and then put to test in New York City in a prospective, randomized trial of annual invitation to mammography plus physical examination vs. current clinical practice in the HIP project. The statistically significant mortality reduction from BC in the study vs. control group [3, 9] was confirmed by further updates of the HIP data [10], as well as by a number of subsequent RCTs set up in the period 1976–1991.

Up to 14 RCTs [11] could be considered, the total number depending on counting trials with two parts (Malmoe, Swedish Two-County, Canada I and II) as separate studies or not and on exclusion criteria for one or more trials on different motivations, either of design or of their quality. As a consequence, meta-analyses and systematic reviews of RCTs, the most common gold reference for directing decisions on screening policies, may vary in their conclusions mainly due to the quality criteria for selection of trials to be included in the review process. More commonly, eight big RCTs are considered, and seven are actually included [7, 11], since all reviews agree to discard the Edinburgh trial on major unbalances in the randomization process [12].

The seven trials considered are the HIP study, started in 1963; the Malmoe trial I, started in 1976; the Swedish Two-County (STC) study (Kopparberg arm, started in 1977, and Ostergotland arm, started in 1978); the Canada I and II (CNBSS), started in 1980; the Stockholm trial, started in 1981; the Gothenburg trial, started in 1982; and the UK Age trial, started in 1991.

It is remarkable that, beyond all the many differences among the trials in design, technicalities (e.g., number of mammographic views), intervals between screening rounds, age groups involved, duration of follow-up, etc., meta-analyses tend to converge on an estimate of around 15–20% relative risk (RR) reduction in BC mortality for women invited to MS vs. the non-invited.

The Marmot report [7] may rightly be considered as the most balanced among the recent highest profile reviews, with regard to the MS debate, coming from a group of independent experts, selected and nominated by the UK authorities on the basis of their knowledge and on the absence of any personal involvement in the dispute. These authors recognize a 20% mortality reduction from BC associated with invitation to screening. They summarize their findings in a table that we reproduce in a simplified form as in Table 17.1, for ease of reference and discussion.

On the other hand, one might regard the series of meta-analyses from the Cochrane Collaboration, as the most pugnaciously critical of MS. Originated from a commission back in 1999, they were first published in 2001 and revised a number of times to the latest review in 2013, to which we now refer as the Nordic Cochrane review (NCR) [11]. These authors consider that the only three trials with "adequate randomization," i.e., Canada, Malmoe, and UK Age trials, did not show a significant reduction in BC mortality, with a RR of 0.90 (95% CI: 0.79–1.02). They recognize that the other four trials that they considered of "suboptimal randomization" showed a significant RR reduction of 0.75 (95% CI: 0.67–0.83). It must be remarked though that the quality evaluation as proposed by the NCR has been substantially subverted by the more balanced review of the UK Independent Panel [7]. The RR for all seven trials in the NCR was statisti-

Table 17.1 Breast cancer mortality reduction in RCTs of mammography screening

Study, date of start	Age group	RR	95% CI	Weight (%)
New York, 1963*	40–64	0.83	0.70–1.00	16.9
Malmoe I, 1976*	45–69	0.81	0.61–1.07	9.5
Kopparberg, 1977	38–75	0.58	0.45–0.76	10.7
Ostergotland, 1978	38–75	0.76	0.61–0.95	13.0
Canada I, 1980**	40–49	0.97	0.74–1.27	10.2
Canada II, 1980**	50–59	1.02	0.78–1.33	10.2
Stockholm, 1981*	39–65	0.73	0.50–1.06	6.0
Gothenburg, 1982	39–59	0.75	0.58–0.98	10.7
UK age trial, 1991*	39–49	0.83	0.66–1.04	12.8
Overall		0.80	0.73–0.89	

A meta-analyses after 13 years of follow-up, based on the Cochrane [11] and Marmot reviews [7] (modified)

RCT randomized controlled trial, *RR* relative risk, *CI* confidence interval

*Studies falling short of statistical significance and/or RRs between 0.80 and 0.90

**Studies with no statistical significance and RRs beyond 0.90

cally significant at 0.81 (95% CI: 0.74–0.87). The NCR concludes that assuming a 15% reduction in BC mortality with MS, one would need to invite 2000 women throughout 10 years to save one life.

Duffy et al. [13] argue that the number needed to invite is not the proper measure, since it will be heavily influenced by the attendance rate of the population; they recommend the number needed to screen (NNS) to prevent 1 BC death, as a more adequate measure of MS benefit. They work on this and make assumptions about a UK scenario. After correction for the actual participation rate to the UK screening program of 77%, starting from the Cochrane value of 15% mortality reduction in the invited women, they come to an estimate of 257 NNS in 10 years to prevent 1 BC death, as compared to the 2000 needed to invite in the Cochrane estimate. It is opportunely remarked that the very low estimate of absolute benefit in the Cochrane review derives from unduly restricting the benefit analysis to a 10-year period and from their selection of trials dominated by the younger (below 50) age group, which has considerably lower absolute mortality. Applying the same reasoning, corrections and NNS to another major recent review by the US Preventive Services Task Force (USPSTF) [14], Duffy et al. come to a similar result of 193 NNS to prevent 1 BC death. They insist that expressing results relative to the same denominator, with the same follow-up lenght, referring to absolute mortality rates, and applying them to different published reviews - we end up with absolute measures of benefit of the same, relevant order of magnitude. This supports the concept that the so-called controversy on BC screening is to a large extent an artificial one.

The NNS idea refers to the underlying problem of trials reporting benefits of invitation (intention to treat analysis), rather than an actual screening. Higher mortality reductions are expected in women actually attending screening; still it is difficult to say by how much, since different background risks may be involved due to selection bias. Women who attend screening are as such representative of a health-aware portion of the population that might gain extra benefits (beyond those conferred by MS) from the attitude that makes them keen to seek medical support, whenever needed.

One way to tackle this theme would be to consider the RCT evidence as the extremely reliable proof on which to base health policies and screening recommendations. It should be reminded though that the trials tested the impact of invitation to screening on BC mortality. As for the benefit expected for a woman actually attending screening, RRs should be best derived from service screening mortality estimates of attenders vs. nonattenders (see next paragraph).

It is also interesting to consider the USPSTF meta-analysis stratified on different age groups. The USPSTF estimates BC mortality reductions of 15% in the 39–49 age group, of 14% in the 50–59, and of 32% in the 60–69. One might consider that these differences could be determined by the well-known detection limitations of mammography in the denser breasts of younger women. Yet, it is worthy of note that the USPSTF estimates of the two younger age groups very closely resemble the Cochrane analysis of the so-called "adequately randomized" trials, among which only the smaller Malmoe trial includes a portion of women over 59 years old. In fact, it is the relative weight of the Canadian data that do not include the over 60 age group, to introduce a powerful bias.

At this point, some special remarks are warranted on the disgraceful impact of the Canadian National Breast Screening Studies (CNBSS) I and II on the screening debate.

The Canadian Contamination

The CNBSS was set up in 1990 as a thoroughly designed, ambitious project, into which enormous energy, resources, and good will were invested. It ended up as a huge amount of significantly flawed data that should not be considered any more in meta-analyses of screening trials. The fact that these data [15, 16] and one recent update of the same, based on a 25-year follow-up [17], have been widely considered in reviews and referred to contributed extensively to building up and maintaining the artificial controversy on MS. This process may be defined "the Canadian contamination." Instead, it has to be clearly stated that these Canadian results lack methodological value and should not be relied upon for evidence-based conclusions [18]. A quick glance at Table 17.1 suffices to show the CNBSS trials as the flagrant outlayers, showing no hint of benefit, as compared to the other seven studies, whose RRs range from 0.57 to 0.83. It is sadly ironic that the outlayer studies, with a flawed evidence base, should have cast their shadow on a wealth of scientifically sound data from so many other researchers.

It was immediately after their original publication in 1992 that a flourish of critics exploded in the scientific literature. These have obviously been resparkled after the Canadian follow-up article was published in 2014 [17]. In a recent paper by Heywang-Köbrunner et al. [18]—to which we refer the reader for an extremely detailed analysis of the debate and for punctual references—a systematic search on this topic yielded close to 300 articles, 70 of which were deemed of special interest. These articles split in two similar parts of 33 "defending" papers, mostly authored by the original directors of the study vs. 37 "critical" articles by a much wider, representative group of researchers.

The long series of critiques to the Canada trials fall in two main fields:

(a) Technical/clinical quality issues
(b) Methodological/management issues

Both are extensive; however, the methodological/management points are overwhelming.

Group (a) critiques were mainly on the quality of mammography. Quality of images (low contrast resolution, insufficient sharpness, and over- and underexposure) and of positioning was so heavily questioned that many external expert reviewers resigned from their position in the trial on claims of unacceptably poor standards and of their corrective measures not being taken into proper consideration. Also the interpretation of films was criticized as some readers had insufficient training and many obvious cancers were missed. Although quality issues must have played a role in the final results, the core reason why the CNBSS trials should not be considered eligible to be reckoned in meta-analyses of RCTs has to be found in the other group of arguments.

Group (b) issues (the methodological/management problems) are indeed conspicuous. The study design has often been described (most prominently by its authors) as ideal, due to randomization being carried out at the individual level. Conceding that, in principle, individual randomization would be preferable over cluster randomization, what in fact matters is the quality of the process. Most reviewers, among them the UK Independent Panel, recognized that cluster randomization produced significant biases in the Edinburgh trial, and on that basis, they excluded it from meta-analyses, but not in the STC trials, where cluster randomization did not result in relevant unbalances, so that the same reviewers agree to consider this study as soundly evidence based, eligible to be included in reviews.

On the other hand, the randomization process blatantly failed in the Canadian trials, as so apparently shown by the disproportionately large number of participants with late-stage cancer in the mammography arm at the first round [17–19]. Indeed, soon after the first CNBSS publication, the observation of the heavily unbalanced distribution of advanced cancers in young women was supported by a series of reports [20–23] on various contradictions to the initial study design. It was reported that randomization was performed at certain sites after a clinical breast examination, blinding was not consistently warranted, and various easy possibilities of subversion existed and could be done in practice. The motivation for this would have been—in good faith and with no fraudulent intention—to guarantee that significantly symptomatic women would be offered a mammogram. It is a recognized fact that at one time the coordinator of one unit was removed because of suspected subversion of the randomization.

The weak defense of the CNBSS investigators has eventually to face the striking fact that among the first round of younger women, 19 advanced cancers were allocated to the screening arm vs. five in the control arm. Also, eight women in the screening arm vs. one in the control arm had previous history of BC. It is clearly preposterous on the investigators' side to argue that a long list of other variables was perfectly balanced in the two study arms, when the most clinically significant variable, i.e., late-stage BC at first round, was so heavily unbalanced.

The Canadian update itself [17] that has been widely publicized to the scope of discrediting the benefits of MS does in fact supports the contrary view. In that paper, deaths from BC detected at year 1 of the study were double (52 vs. 26) in the mammography arm vs. control arm, a fact that is the obvious consequence of flawed randomization, as shown by the exceedingly unbalanced number of late-stage cancers.

It is telling to quote the authors' own words: "it has been suggested that women with a positive physical examination before randomization were preferentially assigned to the mammography arm. If this were so, the bias would only impact on the results from BC diagnosed during the first round of screening … However, after excluding the prevalent BC from the mortality analysis, the data do not support a benefit for MS (HR = 0.90, CI 0.69–1.16)."

This passage is so important as (1) it implicitly concedes that preferential assignations might indeed have happened within the trial organization and (2) recalculates HRs for only incident rounds of the trial demonstrating a clear drop in HRs. At this point, the authors, rather than expressing this as it should be, i.e., as a shocking 50% difference from the infamous HR value of 1.47 in the prevalent round of the trial (explainable only by subverted allocation) to a promising HR of 0.90 for incident rounds, prefer to highlight the fact that this value still shows a benefit of no statistical significance. The point is that it does suggest a benefit that might have been significant (a) in a high-quality screening service, as compared to the low mammography quality documented in the trial setting, and (b) in a more powerful study design or within a proper meta-analysis that should exclude the biased prevalent round data of CNBSS.

It is beyond the scope of this chapter to further discuss a number of questionable points in the CNBSS studies that contribute to make their results definitely not applicable to quality-assured screening programs. Just in passing, these other objections include the following: the studies included palpable, symptomatic cancers; these were in fact not blindly allocated to the two arms; long-term mortality reduction was calculated from a mixed trial participation of one to five rounds during up to 5 years, thus diluting enormously the benefit that would be possibly demonstrated; and recommended biopsies were not systematically performed.

The crucial point is that using these Canadian data, "evidence in the field of BC screening has systematically been omitted, distorted or inappropriately used over the last decades" [18]. Instead, CNBSS data are not applicable to evidence-based results of modern MS.

The Follow-Up Factor

One should regard BC as a group of many different diseases, with an average long natural history. Even back when MS and modern therapies were not available, median survival times for BC patients used to be several years. This explains the fact that no screening trials can show a mortality benefit in the first 2–3 years after their start and also that most benefit has to be reckoned only after many years of follow-up.

Screening could be then compared to an excellent mix of financial investment products. The investor may cash some short-term dividends, i.e., from lives saved after 3–5 years, due to the timely detection of very aggressive, Grade 3 cancers. Most profits will come in the middle term, these being lives saved 6–10 years after detection of Grade 2 cancers, while some long-term returns should be expected from lives saved 11–20 years after the detection of slowly growing cancers.

This consideration justifies the extra mortality reduction that is still evident in RCTs, after the moment when the control group is offered MS: a fact that puzzled many critics of MS, as in the original Cochrane reviews. This phenomenon is particularly well represented in the 29 years of follow-up publication of the Swedish Two-County trial [24] where most of the prevented BC deaths were those that would have occurred over 10 years after the start of screening, from cancers diagnosed in the first 7–8 years of the study, since after that time the control group was exposed to screening.

This supports the principle that in MS, as is the case with other primary and secondary prevention activities, considerable long-term follow-up is necessary for a full appreciation of the benefits involved. In a RCT setting, most benefit is to be expected more than 10 years after the trial starts, from cancers diagnosed in the first 5–10 years (recruitment period), depending if and when the control group is offered screening after the study recruitment phase.

Failure to fully appreciate this concept has led to many inconsistent or weak analyses and meta-analyses and to a substantial undervaluation of the merits of screening.

The importance of prolonged follow-up times will be shown for the observational studies, in the following paragraph. As to RCTs, implications are also important, e.g., when one considers the latest updates of the UK Age and Gothenburg trials [25, 26], both showing significant benefits from screening after follow-up times extended to 17 years, also in younger women (and provided one restricts the UK analysis to cases diagnosed in the intervention phase).

A similar pattern was demonstrated in an overview of the Swedish RCTs [27] that, restricting the analysis to women randomized when 40–44, demonstrated a 15% reduction in BC mortality at long-term (over 14 years) follow-up. In this overview, benefit increased up to 12 years after randomization and was then maintained.

Conclusive Remarks on the Efficacy Data from RCTs

When all the evidence in favor of MS is considered and duly recognized, screening opponents come up with another argument (issue d—in the above "provocative sequence"), namely, that RCT results are too old to maintain their validity in the modern setting. This is largely objectable, and we shall come back to this in paragraph three. However, this point could be considered more appropriate for trials where the quality of mammography technique was grossly antiquated with respect to modern standards. If this is probably true for the CNBSS studies that are to be excluded anyhow on other more weighty considerations, it is certainly the case with the HIP study conducted in the 1960s, where the quality of mammography (combined for that trial with clinical examination) did succeed in reducing cause-specific mortality mainly staging BCs down from the big lumps that were the usual case pattern of the time (often T3+ cancers), to some relatively "earlier" cases, but still typically in the T2+ TNM size category. These, as well as the average cancer size of close to 20 mm in the CNBSS studies, are not representative of the practice of modern MS, where a great majority of cases are below the 15 mm size threshold and many within the 10 mm limit.

What is difficult to perceive, and is thus totally unappreciated by non-radiologist, is that the amazing results of the Kopparberg arm in the STC trial gained one special contribution from the extremely high quality of mammography that the lead scientist of the trial, Laszlo Tabar, could achieve in the late 1970s. That is attested by the fact that the standard textbook on mammography remains to date the teaching atlas that Tabar published some 30 years ago and that in its latest edition of 2011 is still based on the original mammographic films of the late 1970s [28]. That quality was already representative of the good results that modern MS programs can attain.

To sum up the substantial evidence on MS efficacy as derivable from many sound RCTs, one could start from the table derived from the UK Independent Panel review (Table 17.1) and adapt it based on the above discussion (Table 17.2)—excluding the New York and the Canada trials and substituting the latest publications of the UK Age trial and of the Gothenburg trial [26, 27], since these capitalize on longer follow-up periods, which were not available at the time of the Marmot report.

In this updated Table 17.2, most trials show a consistent BC mortality benefit for women invited to screening, in the very narrow range of 0.70–0.76, the two slight outlayers being Malmoe (RR = 0.81) and Kopparberg at the other end (RR = 0.58). In this updated prospect, studies of borderline significance (marked with asterisk (*)—in Tables 17.1 and 17.2) account for only one quarter of the review material vs. two thirds in the Marmot meta-analysis.

Table 17.2 Breast cancer mortality reduction in RCTs of mammography screening, revised and updated

Study, date of start	Age group	RR	95% CI	Weight (%)
Malmoe I, 1976*	45–69	0.81	0.61–1.07	15.2
Kopparberg, 1977	38–75	0.58	0.45–0.76	17.1
Ostergotland, 1978	38–75	0.76	0.61–0.95	20.7
Stockholm, 1981*	39–65	0.73	0.50–1.06	9.6
Gothenburg, 1982	39–59	0.70	0.53–0.93	17.1
UK age trial, 1991	39–49	0.75§	0.58–0.97	20.4

Data derived from the Cochrane and Marmot reviews [7, 11], applying a restricted selection of trials (see text) and substituting the latest updates of the UK Age trial and of the Gothenburg trial [26, 27]
RCT randomized controlled trial, *RR* relative risk, *CI* confidence interval
*Studies approaching statistical significance and RRs between 0.80 and 0.90
§RR for cancers diagnosed during the recruitment period of trial (see text for discussion)
Weight was recalculated as a proportion from Table 17.1

In conclusion, the evidence from many RCTs supports a significant BC mortality reduction from invitation to MS consistently in the range of 20–30%, for women aged 39–75.

17.1.3 Service Screening

While well-conducted RCTs provide the most reliable information about the efficacy of MS (issue a), being subjected to fewer biases than observational studies, many questions have been and are still raised about a number of other points including the actual effectiveness of MS in real practice, the potential harms of screening, and a diminished role for MS in the age of modern treatment: these points (issues b–d) will be discussed in the present paragraph.

17.1.3.1 The Effectiveness Issue

Almost immediately after the initial publication of the HIP results in 1971 [3], not only other RCTs were launched in different countries, but also service programs were set up, and their number increased exponentially following the subsequent publications of the newer studies' results. This has led to the present situation where, in many countries, large screening programs have been implemented on a population base as a core component of systematic national health policies for cancer prevention. This is the case for many European nations [29]. Also outside Europe, more and more nations, from Canada to Australia, are already managing, while others are in the phase of starting organized MS projects. In many other places, like the USA, screening mammography is extensively employed outside the organized setting, in a form that has been defined "spontaneous" or "opportunistic" screening.

The diffusion of large population-based MS programs provided researchers with the incredible opportunity to produce observational studies that, when thoroughly conducted, i.e., with a special attention to a long series of methodological traps, brought a wealth of new evidence to support the validity of MS in practice. Observational studies are generally more recent than RCTs and can thus reinforce estimates of the effects of screening, offering a robust sense of closer comparability to actual practice, in the present era of continuing developments in diagnostic imaging and clinical care.

If this is certainly the case, one has to be warned that especially the harsher critics of MS suggest to consider observational studies as more relevant than the RCTs. Such assumption allows them to allege biases and problems of interpretation as a polemists' weapon and offers a chance to come up with unfocused analyses of population data, in order to diminish the rigorous efforts of many other researchers. The fine details of methodology are beyond the scope of these pages, and we again refer the reader to the References for comprehensive discussions and especially to the very knowledgeable, large reviews of pertinent literature as may be found in the Euroscreen supplement publication of 2012 [4, 30–32] and in the Marmot report of 2013 [7].

Yet it is crucial to remark that with observational studies, it is fundamental to stick to the polar star that helps to identify the immensely useful, valid publications, namely, the availability of sufficient longitudinal, individual data, i.e., very long follow-ups (ideally beyond 10–15 years) with the possibility to link a woman's screening history to her cause of death. Articles falling short of these requisites should be considered with the utmost caution, if not discarded altogether, even when published in highly regarded scientific journals. A firm warning has to be made about this continuous flow of articles where all the basic methodological prerequisites are not met. Whenever reading observational/ecological/trend publications that lack individual data and/or long-term follow-up, one should be aware that these papers actually use invalid material to fuel the artificial debate on MS [33–36]. Based on conjectures and extrapolations rather than facts, there is obviously not much chance that the benefit of MS can be fully appreciated. In Broeder's words [30]: "Much of the current controversy on breast cancer screening is due to the use of inappropriate methodological approaches that are unable to capture the true effect of mammographic screening."

In brief, we may consider among the observational studies:

1. Trend Studies

This would be the weakest group [7, 31], comparing BC mortality trends with regard to the availability of MS on a population as a whole rather than on an individual basis. Methodological difficulties are overwhelming with these studies. Problems include the impossibility to attribute BC

deaths to cases diagnosed before or after the screening activity started, to the possible relevant contamination from opportunistic screening even prior to the introduction of screening [37]. Some studies attempted to include more detailed analyses, fine corrections for various confounding factors, and extended follow-up [38, 39] and still estimate MS mortality benefits in a relatively wide range. In general, these methods should be considered of limited value for assessment of screening activities and have in fact been considered not reliable by the UK Independent Panel.

2. Case-Control (CC) Studies

This is the best known methodology, apart from RCTs, comparing the history of screening exposure between women dying of BC and live controls. Such a design yields estimates of relative mortality in compliers to screening invitation vs. non-compliers. This produces the main, well-known problem of self-selection bias, since compliers and non-compliers may differ a priori in their risk of dying from BC [7]. Therefore, researchers typically have to introduce a correction for this bias, whose adequacy may be questioned by critics. The Euroscreen review and selection of the best European CC studies, with exclusion of overlapping data, confirm a reduced mortality benefit of 31% in invited women (OR = 0.69; 95% CI 0.57–0.93) and 48% in women screened (OR = 0.52; 95% CI 0.57–0.83), after adjustment for self-selection.

3. Incidence-Based Mortality (IBM) Studies

In IBM studies all BC deaths in a population are considered if the corresponding BC diagnosis occurred in a time window when the woman had the opportunity to be screened, due to eligibility and invitation [7]. These BC deaths are then compared with corresponding BC deaths from women not having the chance to be invited on geographical (region with no screening program) or chronological (historical, pre-screening data) basis. A meticulous selection of the studies with the strongest design [30, 32] and excluding overlapping publications demonstrated a mortality reduction for women invited to screening of 25% (RR = 0.75; 95% CI 0.68–0.91). When women actually attending screening were considered, the benefit estimate was 38% (RR = 0.62; 95% CI 0.56–0.69). The huge amount of valuable data involved should be emphasized, as well as the substantial homogeneity of the results across the studies under review.

The Euroscreen estimates, as derived from the detailed analysis of a wealth of evidence-based data of service screening studies and on the most scrupulous methods [30–32], show a BC mortality reduction of 25–31% for women invited to MS and 38–48% for women actually screened. These figures reaffirm the large benefit demonstrated by the "old" RCTs also in the more recent, real-life situations of service screening.

To further stress the extreme importance of these service screening studies and the powerfully distracting capacity of those studies that do not comply with the basic methodological prerequisites (individual data/long-term follow-up), we shall now analyze a few instances in some more detail.

As a paradigmatic example, let us consider the Norwegian Breast Cancer Screening Programme (NBCSP) that was launched in 1996 and what different studies have published about its impact on BC mortality.

Kalager et al. [34] in 2010 on the basis of aggregated screening data, and a maximum follow-up time of only 8.9 years, with an IBM approach, conclude that in Norway the availability of MS was associated with a 28% reduction in BC mortality in the screening group as compared with the historical preceding 10-year period. Since a similar, although lower, reduction of 18% in BC mortality was observed also in the non-screening group vs. the historical comparison group, they conclude that only a third of the total reduction could be attributed to screening, the remaining benefit being interpreted as a result of improved treatment within an interdisciplinary team. As is commonly the case, the role of the organized MS experience of the 1980s–1990s in building up the concept of the specialized interdisciplinary, collaborative management of BC that has recently led to the institution of the Breast Units system as an international standard of care is not remarked.

In 2013 Olsen et al. [40] still based on aggregated data and an IBM approach, with a maximum follow-up of 13 years, try to improve on some aspects of Kalager's work, in order to correct possible underlying temporal changes in BC mortality. They conclude that the implementation of the Norwegian-organized screening program was associated with a nonsignificant decrease in BC mortality of 11%. There is again a misleading message in this apparently disappointing summary conclusion. In the first place, it should be emphasized that this result does not represent the impact of MS on BC mortality, i.e., this is not a comparison of screening vs. no screening. Rather, it depicts the impact of building an organized MS program on top of existing widespread spontaneous mammography. In Norway, this was estimated by the authors at around 40% prior to the program. Eventually, one might read the conclusions of this study either in an erroneously diminishing fashion as a "nonsignificant effect of MS" or—more opportunely—as a coherent, promising observation of an "extra effect on mortality from organized screening," as compared to a similar, widespread, non-organized mammography coverage of the population, and this extra effect is perceivable even at relatively short follow-up, still in the recent era of modern treatment. This makes altogether a different picture.

Conversely, the first report of the Norwegian program, which was based on the access to individual screening data

[41] with a maximum follow-up of 15 years, shows a significant, conspicuous 43% mortality reduction from BC (RR = 0.57; 95% CI 0.51–0.64) associated with attendance, after adjusting for several factors, most notably for self-selection bias.

After the previous discussion of the serious perturbation of scientific evidence associated with the publicity of the Canadian trials, it seems relevant at this point to emphasize the results of an excellent analysis of BC mortality in a service MS situation published in 2006 by Coldman et al. [42] on data of the Screening Mammography Program of British Columbia (SMPBC) established in 1988 in Canada. The authors show that MS significantly reduced BC mortality at all ages between 40 and 79. Mortality reduction was 40% for all ages combined (RR = 0.60; 95% IC 0.55–0.65). In women entering screening at age 40–49, the reduction was 37%, after exclusion of mortality associated with cancers diagnosed after age 50. Even after correction for self-selection bias, the mortality reduction was 24% for all ages.

In Italy, a series of valuable publications have been produced over the years by the IMPACT study project, a national research task force based on an extensive database linking BC cases in areas covered by cancer registries to individual screening files. In the IMPACT project, all cases are classified by cause of death and detection method (screen detected, interval cases, never respondent, diagnosed before invitation). From this material, a case-control study [43] assessed BC mortality reduction associated with MS exposure at 45% (OR = 0.55; 95% CI 0.36–0.85), over and above the background access to mammography, thus confirming the important impact of service screening in the Italian health situation. The OR associated with invitation was also significant at 0.75 (95% CI: 0.62–0.92).

In 2013, the IMPACT Working Group produced another study of outstanding importance demonstrating a significant decrease of advanced-stage cancers after the introduction of organized screening in Italy [44]. This represents a central issue in the ongoing evaluations of screening programs in practice and is based on an early indicator derived by the data of the STC trial. As back as in 1989–1992, Tabar et al. [45] showed that the incidence of stage II and greater cancers started to decrease 5 years after randomization and this decrease paralleled quite neatly the decreasing mortality curves in the study, with a substantially stable 30% reduction from 8 years onward. This proves that early diagnosis does interrupt the natural history of BC, and this has led to the proposal of the incidence of late-stage BC as one powerful surrogate indicator of a MS program effectiveness.

Many studies have aimed at assessing this parameter, with conflicting results, some confirming the reduction in advanced cancers [46–50], while others showing stable rates over time [51–54]. The IMPACT Working Group study of 2013 [44] adopts a sophisticated approach in order to tackle the subtle methodological traps that are hidden in a service situation, especially from subgroups of the dynamic target population. In this, at any point in time, there are always subgroups of women whose screening exposure is so short as to have no measurable impact, thus causing a dilution of the screening benefit (in part again a consequence of working with insufficient follow-up times). Among the solutions adopted in this study, there was the exclusion from analysis of women aged 50 to 54 because of screening exposure necessarily below 5 years and reference to pathological tumor size (beyond 2 cm) to define advanced cases, rather than the pN data, in consideration of the substantial stage migrations observed in recent years after the introduction of sentinel node biopsy and improvements in the pathological study of lymph nodes. This study, based on a total of 14,447 incident cancers, was able to show a significant and stable decrease in the incidence of late-stage BC from the third year of screening onward. Incidence rate ratio was 0.81 at years 3–4, 0.79 at years 5–6, and 0.71 at years 7–8. This result is consistent with an effect of MS in reducing advanced cases (which anticipates the effect on mortality) around 20% in the first 3–4 years after the screening starts, increasing to some 30% in the medium term (5–8 years), showing a consistent effect in a real-life situation with data of a screening population of 700,000 women, 55–74 years old, from 700 Italian municipalities.

To further stress the importance of extended follow-up times, one cannot leave unmentioned one large Swedish experience of service screening, where an earlier assessment based on mean follow-up of 8 years [55] yielded a nonsignificant impact of MS on BC mortality of younger women (40–49 years old) with a RR of 0.91 (95% CI: 0.72–0.95), while a subsequent publication on the same material [56], but with follow-up extended to 16 years, gave a strong, significant 38% mortality reduction in the same age group (RR = 0.62, 95% CI: 0.42–0.91).

Another study that deserves a special mention was published in 2011 [57] and represents one among many outstanding contributions from a research group based at the Dutch National Reference Centre for Screening in Nijmegen (in this case, as a joint effort with UK experts). This study investigates the impact of screening from the start of the Nijmegen service screening program in 1975 up to 2008. With a case-referent approach [58], BC death rate was 35% lower in the screened women, in the complete period. What is new to this study is the demonstration of a favorable trend of increasingly strong reduction in mortality over time, attributable to MS, from 28% in the period 1975–1991 to 65% in the years 1992–2008 (OR =0.35; 95% CI = 0.19–0.64). The authors consider the probable role of improvements in the quality of service screening in achieving these results, not only from a technical point of view (i.e., availability of more modern technologies) but also from progressions in quality assurance and special training of dedicated

personnel. Also, the multidisciplinary management of BC and a greater combined effect of modern treatment and early detection are highlighted, as possible causes of this progressively increasing benefit.

17.1.3.2 The Overdiagnosis Issue and the Balance Sheet

Given the massive high-quality data in favor of a relevant positive effect of MS on BC mortality, such as to be eventually conceded even by the harsher opponents, the last decade has seen a new outburst of objections, focused on the alleged harms of screening potentially surpassing the possible benefits. In other words, the question is whether the benefits provided by MS are more substantial than any unwanted effect that it may produce.

This debate has often taken the form of a "balance sheet" of screening benefits vs. the potential side effects of the organized intervention. The major potential harms that are taken into account are false-positive recalls and overdiagnosis.

Other negative effects are generally agreed to carry a negligible weight. These would include the risk of X-ray-induced cancer, estimated at 1–10 per 100,000 in a recent review [59], and the false reassurance, which might entail a delay in BC diagnosis after a negative screening result; this is also considered to have minimal effects [60]. When performing the balance sheet exercise, depending on a series of assumptions and on the reference value considered, as apparent from the simple comparison of Tables 17.1 and 17.2, the final picture can be very different. All in all, the Euroscreen publication of 2012 [61] provides the best reference demonstration to date of a well-devised scenario based on a reasonably weighted evidence base.

Overdiagnosis

Central to this field of dispute, the argument of overdiagnosis has been fueled by many in these last years and has in fact been at the basis of the institution of the special panel of experts in the UK that eventually produced the "Marmot report" [7]. To this, the reader is once more referred for an extensive, knowledgeable coverage of this particular argument, and its many methodological implications, although some caveats, will be discussed in this paragraph.

Overdiagnosis is indeed a momentous subject in screening research and evaluation. It refers to the possibility that anticipating the time of diagnosis before clinical symptoms are apparent will result in a number of cancers diagnosed, which would not have provoked harms in the woman's lifetime, if not detected by screening. The two crucial aspects are the quantification of overdiagnosis and the impact on the woman's well-being of an overdiagnosed cancer.

The major methodological difficulty in estimating overdiagnosis lies in the ability of recognizing the excess incidence due to lead time and separates this from that due to overdiagnosis. The excess "lead time" incidence is in fact a requisite of MS, necessary to allow for early diagnosis and effective treatment. In the absence of overdiagnosis, this increase in BC incidence as women enter the screening program would be balanced by a similar decrease in cancers among older women exiting the program at the upper age limit: this phenomenon has been defined as the "compensatory drop" [62]. Again, this requires either a very long follow-up time in order to be fully accounted for or some well-devised statistical adjustment. The UK Independent Panel, recognizing the utter difficulty of the estimate, takes a conservative position, based on data from only a few RCTs (Malmoe plus the Canadian trials), and considers overdiagnosis at about 5–15% from the population perspective and 15–25% from the individual woman's perspective.

The Euroscreen Working Group [61] starting from a focused review of the literature [63–67] concludes on a more substantiated estimate of overdiagnosis in the range from 1 to 10%.

A recent work by Duffy and Parmar [68] reinforces the need for observations up to 10 years beyond the upper age limit for screening (which means up to 30 years of complete follow-up) in order to compensate for lead time and nullify the pseudo-excess of overdiagnosed cases. This represents one further and very strong caveat against all studies that fail to take into account the very long natural history of BC and the related lead time required in order to cash the screening benefit: such studies would produce inconsistent conclusions if based on nonindividual data and/or too short observation times. Also the need for correcting for underlying incidence trends independent of screening requires estimates and extrapolations. This adds to the difficulties and has been taken by some as an excuse to ignore a problematic issue, in fact ending up with even less reliable estimates. Duffy and Parmar convincingly conclude that previous measures of overdiagnosis are likely to be overestimates. They point to further empirical evidence that overdiagnosis is a smaller problem than generally thought, as can be derived from the TCS, where at 29 years the cumulative incidence was identical between study and control groups [69].

However, they also admit that their estimates include only the invasive cancers, while a substantial part of the overdiagnosis debate involves the possibility that MS could detect a vast number of preinvasive lesions that might never evolve into clinically significant cancers. One very recent study [70] shows that this assumption—and the idea that large numbers of invasive BC would never progress in the absence of treatment—might have no actual evidence base. In this paper, an analysis of data from over 5 million women in the UK screening program showed an inverse correlation between invasive interval cancers and DCIS detected at screening. This association suggests that detection and treatment of DCIS at MS effectively prevent invasive disease.

The balance sheet

The Euroscreen Working Group [61] has created a decision-making scenario where the essential components of the harm/benefit balance could be fitted and discussed in a way that could be effectively communicated to the population involved [71]. Such a setting would also allow for the possibility that updated figures could be inserted and worked up as new evidence should be made available. This scenario considers 1000 women entering MS aged 50–51 and screened biennially until 69 and followed until 79 years (a substantial observation time of 30 years). Based on evidence from European service screening programs, results are expressed as a number of women that need to be screened (NNS) in order to achieve any specific outcome. With this framework, estimates are of 125 NNS to save one life (benefit) vs. 250 NNS to have one overdiagnosed BC and 33 NNS to have one invasive assessment (harms). These results represent a brilliant, honest, scientifically sound collection of data that are intended as a tool that will help a woman who is invited to screening to make an informed personal choice about the implications of participating. To such scope, a narrative was also created to help explain a complex situation, like screening actually entails [61]. Two small European cities are described, with 1000 female residents aged 50–51, where only one city invites women to an organized MS. This results in the outcomes outlined in Table 17.3: over 20 years, there will be eight fewer deaths from BC at the cost of four overdiagnosed cases and a considerable number of false-positive assessments. In this narrative, it is stated that "most of the women participating in screening will have only negative mammograms and, therefore, will have no benefits other than a reassurance about their health status, and only short-term harms from service screening (discomfort, anxiety)."

Arguably, this last point may be considered as a diminishing appreciation of the importance of regular, true reassurance about individual women's health status, with regard to such a high incidence disease as BC. At a closer survey, the picture delineated in the Euroscreen narrative shows some weakness in its aiming at a faithful representation of the health-care scenario in the absence of organized MS. Indeed, BC expected in the population with no organized MS should not be considered to come at no cost, be it financial or from side effects. In the absence of an organized program, women still have breast symptoms; besides that, some of them do have tests in a "spontaneous" screening fashion.

Organized MS involves setting up multidisciplinary specialized units, staffed by dedicated personnel, with special training. It also requires regular quality assurance procedures, monitoring, and evaluation of ongoing activities. Screening guidelines and protocols pay close attention to specificity and require that screening cases come to a definite conclusion after each episode, discouraging short-term repeat examinations, as is common practice in many clinical settings.On the other hand, areas not covered by organized screening tend to be served by non-breast dedicated clinicians, resulting in a higher number of unnecessary examinations, inconclusive test, and less straightforward protocols. This is represented in the comparison of the UK organized screening vs. the performance of spontaneous screening mammography in the USA, as detailed in a study by Smith-Bindman et al. in 2005 [72]. This showed that a slightly higher cancer detection rate in the USA was obtained at the expense of more than double recall rates and surgical biopsy rates. These results are fitted in a scenario similar to the one in the Euroscreen balance sheet. A face-to-face comparison (see Table 17.4) immediately shows that it is totally unfair to suggest that the city with organized MS produces 200 false-positive recalls, thus causing more psychological harms than in a neighboring city with no such program.

A possibly more faithful narrative—to accompany and illustrate a revised form (Table 17.5) of the balance sheet—may be the following:

Consider two small towns where an important group of diseases, namely, breast cancers, because of their clinical implications and very high incidence, cause per se a large burden of anxiety in the female population. In one city an organized, controlled, specialized program offers women the continuing reassurance of well-managed periodic tests, significantly cutting back the mortality rate from the disease, at the cost of a limited number of overdiagnosed cases. Participating in such program would also confer these women a reduced burden in terms of false-positive assessments, less psychological harms from too frequently repeated examinations with no conclusive diagnosis, as compared to the neighboring city where such program and all the related skills, organization, protocols, and

Table 17.3 Harm/benefit balance sheet for organized mammography screening of 1000 women[a] from the Euroscreen Working Group 2012 [61], modified and expanded

Outcome	For every 1000 women screened for 20 years	NNS
Number of BC diagnosed	71	14
BC mortality reduction	8	125
Over-diagnosed BC	4	250
False-positive (FP) tests, of which:	200	5
– FP recalls, with non-invasive assessment	170	6
– FP recalls, with invasive assessment (biopsies)	30	33
Reassurance of true negative cases (all rounds)[b]	729	1.4
Equity of access to high quality health care[b]	1000	1

BC breast cancer, *NNS* number needed to screen
[a]Women entering screening at age 50, screened biennially until 69 and followed until 79
Mortality reduction was adjusted for self-selection bias
[b]Original entries

Table 17.4 Harm/benefit balance sheet for mammography screening of 1000 women over 20 years in an organized European setting compared to a US estimate for spontaneous screening, modified and expanded from [61, 72]

Outcome	For every 1000 women screened for 20 years	
	Euroscreen [61]	US [72]
Number of BC diagnosed	71	55
BC mortality reduction	*8*	*8*[a]
Overdiagnosed BC	*4*	*4*[a]
False-positive (FP) tests, of which:	200	694
– FP recalls, with noninvasive assessment	170	553
– FP recalls, with invasive assessment (biopsies)	30	142
Reassurance of true negative cases (all rounds)	729	306
Equity of access to high-quality health care	1000	Not applicable

[a]Mortality reduction and overdiagnosis arbitrarily assumed to be of the same magnitude as in the Euroscreen estimate
BC breast cancer

Table 17.5 Harm/benefit balance sheet for organized mammography screening of 1000 women[a] (current proposal)

Outcome	For every 1000 women screened for 20 years	NNS
Number of BC diagnosed	71	14
BC mortality reduction	8	125
Overdiagnosed BC	4	250
Reassurance of true negative cases (all rounds)	729	1.4
Equity of access to high quality health care	1000	1

[a]Women entering screening at age 50, screened biennially until 69 and followed until 79
BC breast cancer, *NNS* number needed to screen
Mortality reduction adjusted for self-selection bias

quality assurance are not available. The point suggested in the present pages is that the false positives of organized MS should in fact be considered as a protection conferred by screening, being largely inferior in number when compared to a setting of spontaneous, low-specificity clinical and preventive medicine. Hence, balance sheets of harm/benefits of organized screening should not register the false-positive recalls as screening harms. Instead, the true reassurance conferred to the majority of the population, again and again over many years, by an organized program and the equity of access to highly specialized medical care that service screening provide, should stand out among the major benefits of MS alongside the topmost target achievement of reduced BC mortality (Table 17.5). So, the above-quoted statement might be reworded as most of the women participating in screening will have only negative mammograms and, therefore, will have the continuing, long-term benefits of a reassurance about their health status and only short-term harms from service screening (discomfort, anxiety). A valuable communication of benefits and harms of screening to decision-makers, to women, and to the scientific community itself [71] should consider alongside the effectiveness and the limitations of the procedure and the relevance of such factors as trust, gratitude, and convenience that may play an important role in the informed choice to participate. It should be explicit that balance sheets (Tables 17.3 and 17.5) are the product of dedicated professionals. They are bound to set up effective health initiatives and on this basis produce communication tools that can be transparent and honest, but that cannot be neutral. There are other historical merits to be credited to MS. The leading role of the organized MS experience of the 1970s–1990s in building up the idea that there was a need for dedicated professionals with specific education, training, and expertise in BC diagnosis and treatment is rarely, if ever, remembered. The importance of interdisciplinary, collaborative management of BC by experts in senology has been advocated by the screening guidelines, at a time where senology was hardly recognized by most physicians as a field of specialization in its own right. This awareness has greatly contributed to the institution of the Breast Units system as an international standard of care. An important concluding recommendation would then be, when reminding potential harms of attending screening, to give a proportionate emphasis also to harms entailed by not attending the program: larger tumors, worse stage at diagnosis, more systemic treatment, and worse survival.

17.1.3.3 Inconsistency of the "Expired Validity" Issue

It has been shown that RCTs and service screening data proved that MS is valid and effective and that its side effects would be minor with respect to the potential benefits. At this point, the question has been arisen whether early detection through MS still holds its meaning in the new era where very effective treatments have become available and if most of the mortality reduction from BC that has been recently observed should be credited to treatment, rather than screening. This is a reasonable question in itself, but once again the answer is clear: there is substantial evidence that MS still plays an important role in BC management and cause-specific mortality reduction.

Some of this evidence has been already discussed in the above paragraphs. Of special relevance to this point are the service screening studies performed in the last 15 years [30–32, 40–43, 56, 57]. These do show net benefits for women attending MS compared to nonattenders, who still have potentially access to all the advanced treatments available in the regional health-care system. One publication [57] has brilliantly shown that screening not only retains its effectiveness in the recent years of sophisticated oncological

treatment, but in fact it contributes a favorable trend of increasingly strong reduction in mortality over time. This reminds that alongside advancements in therapy, improvement of radiological techniques also come into the picture, enhanced by the virtuous setting of quality assurance, dedicated training, and interdisciplinary collaboration in a new Breast Unit arrangement that organized MS contributes to develop.

The intuitive concept that even in an epoch when sophisticated systemic therapies are available, small, node-negative BC as those detected at screening still carry a significant survival advantage, has been confirmed by many.

Of special interest, and largely unappreciated by many physicians, is the demonstration [5] that screening detection of small tumors not only reduces the incidence of lymph node metastases but also prevents the worsening of their malignancy grade.

An Italian service screening study [73] showed an improvement in survival rates by before-after invitation period in an intention to treat analysis addressing the fact that screening changed the pattern of tumor characteristics in the population. Within the same tumor characteristic subgroups, survival was comparable, supporting the hypothesis that the difference in prognosis observed was due to early diagnosis rather than differential treatment or access to treatment.

Other experiences [42] support the idea that notwithstanding the advances of modern systemic therapy, large differences persist in prognosis by extent of disease at diagnosis. One paramount confirmation is from the Swedish experience, where individual counties had the possibility to choose 40 or 50 years as the lower age of screening. This gave the chance to measure the impact of screening in a population aged 40–49 including over 16 million women-years with 16 years of follow-up. The significant 29% decrease in BC mortality that was demonstrated for women who attended screening (RR 0.71; 95% CI 0.62–0.80) occurred in a country with uniform treatment guidelines. This proves that this mortality reduction was achieved in addition to the benefits of modern therapeutic advances [74].

It is clear that both early detection and modern treatment have merits in achieving the long-awaited for reduction in BC mortality: it would then probably be a much better way to look into the future to recognize the mutual enhancing power of the two, as early detection allows for more refined treatment options and for the adjuvant therapies, both medical and radiation based, to achieve extraordinary results in disease control. In other words, rather than keeping up a long sequence of futile controversies, it could be more advantageous to devote resources to a very appropriate topic for research: how early detection might or should change the treatment of some subgroups of BC.

17.1.4 Evolution

A positive evolution of BC screening has to build on the clear appreciation of what can already be achieved through the "classical" population-based programs. Physicians, healthcare providers, and the population alike have to understand that MS contributes a significant reduction in BC mortality and represent a major achievement and a public health intervention of demonstrated feasibility and cost-effectiveness. Future developments of screening should prove not only their absolute efficacy but also their feasibility and sustainability in terms of incremental cost-effectiveness, in order to guarantee that the new policy should not put at risk the regular management of the existing MS programs.

To date, screening has been implemented on the two strongest risk factors for BC, i.e., sex and age. However, in this epoch of personalized medicine, the concept of tailoring BC screening to different levels of risk has gained increasing interest. Mammography has been regarded as the most suitable test for screening, due to the evidence available, its reasonably high sensitivity and specificity, and low cost. It is important though to be aware of the limitations that a single screening tool entails and that while alternative breast imaging techniques have been around for decades, recent advances in digital-based diagnostic devices and information technology (IT) have widened the spectrum of imaging possibilities.

Keeping in mind the big caveats regarding (1) evidence of efficacy, (2) incremental cost-effectiveness, and (3) sustainability, one might think about screening evolution, apart from the special policies already envisaged for the population at the highest risk (the theme of the following chapter) according to the three main pathways:

(a) Tailoring the screening process on the basis of different levels of risk (low to intermediate)
(b) Introducing new screening tools (technological evolution)
(c) Increasing the effectiveness through improvements in the overall quality of the process

17.1.4.1 Tailored (Risk-Based) Screening

This involves the idea of offering customized screening policies on factors influencing the risk and/or the performance of the intervention, such as (1) age, (2) breast density, and (3) other personal risk factors. The assumption is that benefits and harms/limitations of screening vary according to BC risk, so that such tailoring of interventions may optimize their balance.

(1) Age—Besides sex, age has always been identified as the main risk factor for BC. All MS projects have been tar-

geted to those age groups where the general consensus recognized the optimal cost-effectiveness balance; these are most commonly the 50–69 years old women.

Younger and older women have always represented a subject for discussion, and in the past, there was a major debate over the appropriateness of offering MS to women in their 40s.

Arguments against screening the 40–49 years old included the lower incidence (and mortality) and the predictable lower efficiency of the screening test due to the limitations of mammography in denser breasts, both of which contributed to the lower mortality reduction observed in the RCTs. Recent data have clearly demonstrated a relevant impact on mortality also in these younger women, when offered MS. This is unequivocal in studies that can provide extended follow-up [56, 74, 75]. As to incidence, the major, abrupt increase in most western countries is obviously at the 40–44 age group, when incidence exceeds 100 cases per 100,000 women per year. Women diagnosed with BC when 40–49 account for a significant proportion of the BC mortality, in fact similar to that attributable to 50–59 and 60–69 years old women [76]. This leads to the conclusion that there is no scientific reason to exclude this age group from a screening program, beyond issues related to resources and feasibility.

Another important point to consider is that life expectancy at birth has in many countries surpassed 80 years for the female population, and for women aged 69 (the upper age target for most programs), life expectancy may exceed 15 years. This implies that stopping invitation after 69 is no longer adequate. Since diagnostic capabilities of mammography in older women are particularly good, and screening efficacy up to age 74 was proven by RCTs, also the optimal upper age limit for screening should be carefully discussed. In 2007, the Italian Society for Breast Cancer Screening (GISMa) produced a consensus document [77] that envisaged the possibility to extend screening to age groups 40–49 and 70–74, where sufficient resources were available. This has in fact been implemented in some Italian regions. A similar strategy of extended screening beyond age 70, based on self-referral of women interested, is in practice in the UK. Sweden, the home to most of the historical RCTs, has been and probably still is the country with the widest age span covered by screening: women aged 40–74 years are offered screening in many Swedish counties as opposed to 50–69 years of age in most other nations.

Another aspect strictly connected to age is the interval between screening rounds. The evidence base for current protocols lies mainly in the results of the RCTs. Considering the high proportional incidence of interval cancers in the second year after screening in the age subgroup 50–54, the Swedish option of screening ages 40–54 every 12–18 months and switching to the 18–24 months interval for ages 55–74 is arguably a better solution than the 24 months interval, starting at age 50 that is adopted by most screening guidelines worldwide. Availability of financial resources still remains one background decisive factor in determining these policies.

(2) Breast density—Breast density, being both a risk factor and a determinant of lower performance for mammography, has been the most discussed criteria to develop customized screening strategies. Many studies and proposals have been produced on this subject, actually resulting in very limited practical achievements until very recently. The subject remains extremely complex, and some issues are still to be clearly defined. Different patterns and composition of breast densities exist; the relation between density and cancer risk needs to be further understood, although it is clear that high mammographic density decreases sensitivity and the positive predictive value (PPV) of mammography, resulting in more interval cancers.

The introduction of digital mammography has already modified this situation to some extent, although the major advances are expected from the introduction in screening protocols of more modern, tomographic imaging techniques, like digital breast tomosynthesis (DBT) and automated whole-breast ultrasound (AWBU).

In recent years, modern digital technology has also made available softwares that can automatically calculate breast density values; these softwares may contribute to higher reproducibility in the classification of density levels. These measures are then used alongside personal risk factors in the definition of statistical models of BC risk. However, a precise definition and a consensus on optimal thresholds and statistical models are still lacking. In the USA, a specific legislation has made it mandatory to inform women about their breast density and the limitations of mammography in dense cases, so that women may decide to have additional examinations. From an organized screening perspective, before additional diagnostic techniques or modified protocols do not prove cost-effective, it would be questionable to stress communication on this issue, which is also generally exaggerated by the use of relative rather than absolute risks.

(3) Other risk factors—Other personal risk factors have been considered, including personal history (previous BC diagnosis or atypical hyperplasias), family history of BC, socioeconomic status (SES), comorbidities, etc. More recently, milder degrees of hereditary susceptibility to BC have been considered, as those related to the study of single nucleotide polymorphism (SNP) [78, 79].

As the overall risk cannot be calculated as a mere sum of different risk factors, it will be essential to develop and validate efficient prediction models. The availability of more sophisticated IT support will probably provide powerful tools and play a decisive role in the advancement of this line of clinical research, also through sophisticated modeling that may con-

tribute to the design of risk-stratified forms of screening, where a better balance between costs, harms, and benefit could be achieved offering adapted programs to different groups of women [80, 81]. In this framework, costs and harms may be contained also reducing screening offer to women at lower risk.

In summary, risk-based tailored evolutions of MS are at hand where revision of the age limits and frequency (1) of screening are concerned. As for factors in points (2) and (3), the general situation is that offering more intensive (or also less intensive) screening, based on one or a combination of the above factors, might indeed result in a qualified improvement in the risk/benefit balance. However, more research and clear data are warranted, as the underlying concept states that marginal gains in effectiveness have to be proven, and the big caveat remains about creating increasing motives of complexity that could eventually detract from the practical management of the screening system.

One major challenge for the future would be to devise strategies where risk-stratified screening would be offered in combination with primary prevention measures, targeting modifiable risk factors, like obesity, through interventions on diet, lifestyle, etc.

17.1.4.2 Introducing New Screening Tools (Technological Evolution)

This is the most promising pathway for BC screening evolution, given the development over the last decade of very promising, new imaging tools, sharing two common denominators: digital framework and tomographic technology. Indeed, tomography-based imaging ideally represents the optimal solution to overcome limitations of mammography in dense breasts. These techniques are (1) magnetic resonance (MR), (2) digital breast tomosynthesis (DBT), and (3) automated whole-breast ultrasound (AWBU). Since these are the subjects of the following chapters in this textbook, to these the reader is referred for extensive discussion and relevant references. At this point, only a very essential-focused comment will be given.

1. MR is by far the most powerful instrument in this series, combining excellent morphologic, three-dimensional representation with functional data. At this moment, however, its use in screening has to be limited to the very high-risk patients, mainly on cost considerations.
2. DBT has the widest literature as a potential new screening instrument. Being in fact a modified version of mammography, its introduction in the screening organization is relatively simple, and a number of studies have proven its ability to increase cancer detection rates in screening settings [82, 83]. Data on specificity are less uniform, yet promising as well. Concerns about the higher radiation dose delivered to the population will be probably overcome by technical developments and especially by the introduction of synthetic 2D images. These should dispense with the need to obtain a double exposure in order to have 2D and 3D images available for the same woman. The main research topic for DBT in MS remains the demonstration of a significant impact on the interval cancer rate. Cost issues are mainly related to the prolonged reading times of the tomographic sequence, rather than to significant modifications in the patient workflow. In fact, the extremely promising diagnostic data and its minor impact on the screening organization have led to DBT being already introduced in some screening programs, within randomized trials or pilot demonstration studies.
3. Automated whole-breast ultrasound (AWBU) takes into the diagnostic field a brilliant combination of the superior ability of sonography to read through the denser portions of the breast with the advantages of an automated procedure that is able to guarantee a more standardized coverage of the breast volume. Due to the superior sonographic potential in dense tissues (at no radiation costs) and hence also a powerful integration with mammography, this technique carries the potential for a more relevant diagnostic contribution than DBT. However, a few studies available to date in the screening setting, while confirming the expected very promising detection gain, show a substantial increase in false-positive values [84]. Moreover, the introduction of this technique in the screening context appears to be more demanding, not only for the extended radiological reading times but mainly in terms of radiographers' working time.

Another important contribution to be expected in the near future is the development of dedicated CAD (computer-assisted diagnosis) systems that will reduce the costs involved with the reading times of long series of tomographic images, be it DBT or AWBU.

17.1.4.3 Optimizing Existing Programs

It has been strongly represented how MS produces substantial benefits to the population in terms of cause-specific mortality reduction, and it has been discussed in the harm/benefit paragraph that an organized screening program provides significant advantages in terms of cost-effectiveness as compared to a spontaneous setting [72]. This reinforces the idea that optimization of the available system would be a rewarding field of evolution. Also in this field, the digital revolution of the past decades offers a number of new, interesting possibilities.

A recent, extremely detailed comparison of the costs involved by an organized service screening system [85] demonstrated significant savings both for the health system as a whole and from the women's point of view. The cost of mammography in a non-organized setting was more than

double compared to the organized program. Outside organized MS, social costs would also be higher, as those related to time lost from work, travel to the screening unit, telephone calls, administration costs, etc.

Moreover, the practical support provided to the female population by the organized setting, from the letter of invitation onward, contributes to its capacity to reach women in the lower socioeconomic categories, thus reducing inequalities in breast cancer survival. In one study [86] the lower survival rates in less-educated women before the launch of the organized MS disappeared completely in the age group invited to screening. The current design of MS has one major strength in the availability of a complex organization that embraces such aspects as detailed shared protocols and guidelines, quality assurance and audit systems, continuous evaluation, and feedback on the results to stakeholders. This is typically represented in the European Guidelines for Breast Cancer Screening [87] and in similar documents produced at the national or regional level in many countries.

Some crucial points that might be developed (and greatly gain from the introduction of digital mammography and the IT support) include:

1. Expanding the monitoring system, from the general program/unit level to the level of the individual operator, with regular personalized feedback on professional screening performance (e.g., recall rates, cancer detection rates), in order to allow for timely educational refreshments where needed. It is important that among the many performance indicators [87, 88] the most relevant will be selected for their special value. Interval cancers, representing a failure of the procedure, should be fully monitored to evaluate screening performance. The radiological revision of pertinent mammograms is a valuable tool of internal audit and a valuable occasion for training and continuing education of the screening radiologists. However, complete data on interval cancers may be difficult to collect. Large cancers (20 mm or more, i.e., T2+) that are screen detected at subsequent rounds represent an equally strong indicator of screening performance and (when combined with the T2+ interval cases) are the best early surrogate indicator of screening impact [5]. Screen-detected T2+ cancers are immediately available at the screening unit, so that their radiological revision would be more easily feasible than reviewing interval cancers—while also their educational value would be substantially similar [89]. As to the evaluation of screening performance and impact, analysis of the pathological size distribution of all BC in the population exposed to screening, expressed as absolute rates rather than percentages, should be regarded as a cornerstone.
2. Recognizing an enhanced role for dedicated education, investing on specialized courses and practical training of all the professional figures involved in the screening process, with a special emphasis on radiographers, radiologists, and pathologists. Specialized education is in many countries largely neglected, while it may probably result in the most rewarding field of investment in order to optimize screening cost-effectiveness. This process should routinely envisage the funding of National or Regional Reference Centres for Quality Assurance and Training for Breast Cancer Screening. The importance of having access to Expert Screening Training Centres is confirmed by the long-lasting experience of the Dutch National Training Centre in Nijmegen, as well as by the Swedish experience. This is effectively represented in one service screening study [90], where organized programs conducted in dedicated centers could consistently achieve mortality reductions at least as high as those observed in the RCTs. This achievement was built on the cooperation of screening centers in seven counties across Sweden, with the expert support of the leading researcher of the STC trial. Expert Reference Centres would represent the ideal site to set up and coordinate relevant research, as the Nijmegen (NL) and Falun (Sweden) experiences confirm.
3. Promoting innovative research taking advantage of the multidisciplinary context of screening. Research should be focused on the key issues of screening evaluation and risk customization. Besides that, it would be most appropriate to exploit the screening setting to foster research based on a radio-pathological cooperation. Improved standard pathologic techniques are to be implemented in order to create a better mutual understanding of the clinical significance of screen-detected lesions. Large-format histologic sections have already proven their value [91, 92] and supported the need for improved pathologic terminology that should reflect the site of origin of the lesions [93]. The integration of imaging morphology into the TNM classification of the in situ and 1–14 mm invasive tumor size range would represent a major advance. There is a considerable potential of mammographic tumor features alongside classical pathological and modern molecular prognostic factors to improve the outcome prediction of BC subgroups [94, 95]. Such radio-pathological synergy could enable the multidisciplinary team to better distinguish the less frequent subgroups with the highest fatality [94] among the small invasive cancers, thus allowing for setting up clinical trials that may identify the more successful, targeted treatment. For the majority of screen-detected, monofocal, small invasive cases that belong to the better mammographic and pathological prognostic groups [96, 97], the current use of adjuvant treatment might be reevaluated through more pertinently designed trials. This research cooperation may

eventually enable many women to forego some of the current adjuvant therapeutic regimens, without compromising their survival and avoiding the hazards of overtreatment. Finely tailored treatment protocols, based on a fuller appreciation of different parameters of tumor characterization, should make negligible any concern over the overdiagnosis of the more indolent cancer cases.

17.1.5 Discussion

The clear scientific evidence on the efficacy of MS as derivable from RCTs and its effectiveness in reducing BC mortality as confirmed by more recent studies conducted in the routine service screening situation have been reviewed and highlighted.

It has been shown how benefits achievable through MS are substantially undervalued.

This is not only the case with a number of skeptical authors, often on the basis of methodological flaws in their arguments. Also some screening advocates appear at times not to fully appreciate the size of the benefits entailed by organized screening. This can derive from:

1. The unjustified consideration paid by many to some large yet scientifically unsound studies.
2. The incomplete appreciation of many experts of the clinical peculiarities of breast tumors: especially their wide inter- and intra-tumor heterogeneity, extremely long natural history of many cases, and the concept of progressive dedifferentiation of BCs. Hence, it is not fully appreciated how MS benefits cumulate over very long times. Some screening dividends of lives saved are cashed as soon as 3–4 years after the timely detection at screening of aggressive cancers, while dividends of lives saved from more indolent cancers might still be cashed 10–15 years after screening detection. The most recent updates of the well-conducted observational studies of screening service, with the longest follow-up times, are wanted to gauge the full effect of MS on mortality (the screening dividend) and should be given prominent attention in the scientific debate. The same applies to the long-term follow-up of the best RCTs.
3. Screening harms related to false-positive recalls are unduly emphasized. It has been illustrated that the limited rate of false-positive recalls in population-based, organized screening is in fact a protection vs. the much higher rates observed in non-organized settings.
4. Neglect of the immense human and social value of MS and the diffuse, continuing real psychological reassurance it provides to the vast majority of true negative women.
5. Insufficient appreciation of the value of equity in the high-quality health-care access provided by organized MS.

It is important to state at this point one rarely, if ever remembered merit of screening. This is the leading role of the organized MS experience of the 1970s–1990s in building up the idea of a need for dedicated professionals, with specific education, training, and expertise in BC diagnosis and treatment. The importance of interdisciplinary, collaborative management of BC by experts in senology has been advocated by the screening guidelines at a time when senology was hardly recognized by most physicians as a field of specialization in its own right. This awareness has greatly contributed to the institution of the Breast Units system as an international standard of care.

There are also important, well-known limitations of cancer screening with mammography.

The lower sensitivity of mammography in dense breasts and—more generally—the traditional use of a single diagnostic tool for the early detection of a complex variety of clinical entities are obvious weaknesses. Although the use of a single test is motivated by evidence of impact, practical feasibility, and competitive cost-effectiveness, intelligent research has to be promoted to open the way to new protocols that take advantage of complementary imaging methods. The recent availability of such sophisticated technologies as DBT, AWBU, and MR will definitely accelerate this evolutionary process. The combination of the newest imaging methods with the powerful support provided by the modern IT systems is due to create a winning environment. Specially developed new CAD systems will help tackle problems related to the longer interpretation times implied by the tomographic techniques. The digital support will also play a role in the form of improved monitoring, evaluation, and educational tools, e.g., mammography test sets for training. Given the limitations of its current format, MS will have also to consider risk-based, customized screening policies, in order to maximize the cost-effectiveness of the system and the harm/benefit balance of the procedure.

So, future evolution of screening should be built on the organized setting of MS: introducing new diagnostic technologies, improving on the stratification of women and the way screening is offered (tailoring), making the most profit from modern IT technology support (simulation models, CAD, etc.), and threading along the main road of the specialized multidisciplinary units, where different specialties work together to optimize the synergies of diagnosis and treatment. Evaluation could be optimized, working on the most significant early indicators of performance (as T2+ cancer rates), refined to the individual operator level, and combined

to a more efficient system of feedback. Optimization should also be pursued of the information provided to physicians and the population and the communication tools.

The prominent importance of dedicated, specialized education, training, and research should be recognized and adequate resources provided. The organized screening framework represents an exceptional resource for producing applied research of the utmost scientific level, at competitive costs. One foremost topic of integrated research would be the innovative rethinking of the pathological classification of breast diseases, to be built on a strict collaboration of breast pathologists and screening radiologists. This new perspective could bring about a change in the fundamental concepts of BC treatment, making it negligible the concerns about screening overdiagnosis.

Conclusion

Implementing, expanding, and keeping up large, high-quality, population-based screening programs should be considered a needful strategy in order to best capitalize on modern treatment advances. In the future context of preventive medicine, innovative strategies may be devised aimed at combining risk-stratified screening with actions of primary intervention targeting modifiable risk factors. Futile controversies on the respective roles played by early detection vs. modern treatment should be abandoned, in favor of a shared awareness that these two major innovations enhance each other's benefits and of research projects on the theme of how early detection through screening might and should change the treatment of breast cancer.

17.2 High-Risk Population

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Abstract Although breast cancer (BC) is mainly a sporadic disease, about 15% of cases are clustered in families at increased incidence. Gene mutations with autosomal dominant inheritance confer a 50–85% cumulative lifetime risk (LTR) and account for about 5% of BCs; about 50% of hereditary BCs are associated with BRCA1/2 mutations. In high-risk (HR) women, mammography has a too low sensitivity (29–50%) to be used alone as a screening tool. Nonrandomized studies showed that contrast-enhanced magnetic resonance imaging (CE-MRI) largely outperforms mammography and/or ultrasound in detecting asymptomatic BCs in HR women, reaching a sensitivity higher than 90% and a positive predictive value higher than 60%. In 2007, the American Cancer Society issued recommendations in favor of *MRI as an adjunct to mammography* for screening women with 20–25% or greater LTR, including those with a strong family history of BC or ovarian cancer or previously treated with chest radiation therapy (CRT). Recommendations in favor of MRI screening for HR women were also issued by other institutions and medical bodies. Studies suggested that MRI screening of BRCA1/2 mutation carriers should not be discontinued over 50 and that an *MRI alone* strategy could be adopted, also considering the higher sensitivity of these mutation carriers to ionizing radiation. Although randomized controlled trials are not allowed for ethical issues, evidence exists in favor of MRI screening to improve patient outcome. In cases of previous CRT, *mammography as an adjunct to MRI* is recommended, because a high incidence of ductal carcinoma in situ with microcalcifications and low neoangiogenesis limits MRI sensitivity.

17.2.1 Introduction

Exactly 30 years ago, in 1986, Sylvia H. Heywang and coworkers reported the first experience about contrast-enhanced magnetic resonance imaging (CE-MRI) of the breast [98]. Notably, only some months before, in 1985, the same author concluded a paper about unenhanced (*non-contrast*) MRI [99] saying that "possible future indications are suggested for selected cases," an elegant way to state that non-contrast breast MRI had no real clinical perspective. Conversely, when, for the first time, a gadolinium-based contrast material had been intravenously injected, "all carcinomas enhanced" and the authors concluded that "preliminary results indicate that MR imaging of breast using Gd-DTPA may be helpful for the evaluation of dense breasts and the differentiation of dysplasia and scar tissue from carcinoma" [98].

This was a turning point which opened a window for breast MRI to enter the clinical practice. At the beginning, even after the introduction of contrast injection, radiologists who pioneered the use of this technology (a name for all, Werner A. Kaiser, who firstly showed the value of dynamic scan for CE-MRI [100]) faced difficulties and distrusts from the established medical community working on breast cancer (BC). Even breast radiologists, who were in those days highly confident with the so-called triple assessment composed by mammography, ultrasound (US), and needle sampling, were not so favorable to MRI. Although mammography was still in the era of film-screen, US B-mode images were distant from today's quality, and needle sampling was mainly fine-needle aspiration, surprisingly breast CE-MRI did not receive a good acceptance.

Breast MRI investigators highlighted that the new method allowed BC identification thanks to its ability to visualize *neoangiogenesis* associated with tumor progression, a completely

new functional imaging approach intrinsically different from the only morphologic evaluation of mammography and US. Physically speaking, two completely different pieces of theory are involved: differences in photon attenuation as an effect of *electronic density* on the X-ray side and differences in nuclear magnetic relaxation times due to the local uptake of the paramagnetic contrast material on the CE-MRI side. Unfortunately, the reference to tumor-associated neoangiogenesis was reminiscent of the old thermography, an approach leading to a false hope for BC diagnosis as it was burdened by a high rate of false negatives and positives,[1] although it is still sometimes represented as a new method [101].

The main criticisms against breast MRI were based on high cost, need of contrast injection, and, above all, an alleged high rate of false positives. A *mantra* arose very soon: *breast MRI has a high sensitivity but a low specificity*. This was due to some papers reporting results of CE-MRI of the breast when descriptors and methods for interpreting breast MRI were still in their infancy. In fact, MRI was firstly considered in the Breast Imaging-Reporting and Data System by the American College of Radiology only in 2003 [102]. Thus, every contrast-enhancing breast finding could at that early stage be considered as suspicious, with the result that small studies often reported low specificity values. Unfortunately, those small studies became the reference against breast MRI.

One clear example of this misleading use of published data is given by the comparison of two papers published in 1993–1994.[2] In 1993, a small breast MRI study from the USA [103], conducted on 30 breasts with 47 malignant and 27 benign lesions, reported a 94% sensitivity and a 37% (!) specificity; these data were included in the Abstract. A year after (1994), a group from Germany, guided by Werner A. Kaiser, reported 2053 cases, 766 with histopathological verification within 2 weeks ($n = 766$) or follow-up control up to 7 years [104]. The title was "False-Positive Results in Dynamic MR Mammography: Causes, Frequency, and Methods to Avoid." Sensitivity was 98%, specificity 97.4%, and PPV 81%. Unfortunately, these results were not reported in the Abstract, thus leading to a strong underestimation of the value of the paper [105]. Looking at the number of citations through Scopus® [106], up to April 26, 2016, the small US study [103] had 580 citations, while the huge German study [104] had only 56 citations. For decades, when researchers reported a range for breast MRI, specificity values, this notorious 37%, the lowest range limit, drew the reader's attention. *Bad news have better legs than good news*.

However, 1993 was also the year of the first report on tumor suppressor BRCA1 gene conferring a high BC risk to women carriers of a deleterious mutation [107]; the identification of a similar role for BRCA2 followed very soon [108]. This relevant new knowledge created the possibility to identify not negligible populations of women having a clearly higher risk of developing BC during their lifetime.

As a consequence, teams of breast radiologists, mostly in cooperation with geneticists, physicists, and other professionals, initiated studies in order to compare the diagnostic performance of CE-MRI with that of conventional imaging (mammography and/or US) for screening high-risk populations. In Italy, we started the discussion in the late 1990s under the coordination of the Istituto Superiore di Sanità, organ of the Italian Ministry of Health, in Rome. For more than 10 years, we guided the High Breast Cancer Risk Italian (HIBCRIT) study for the comparative evaluation of CE-MRI vs. mammography and US for early BC diagnosis among women at high genetic/familial risk. The initial results of this study were published in 2002 [109] and contributed to the initial body of evidence considered by the American Cancer Society for the first recommendation in favor of *MRI as an adjunct to mammography* for screening women with 20–25% or greater lifetime risk [110]. Interim [111] and final [112] results of the HIBCRIT study further contributed to support the use of CE-MRI for screening women with hereditary BC predisposition.

In this chapter, the *high-risk* screening issue is placed in the larger context of the screening debate, and then the evidence in favor of MRI screening protocols for women at hereditary high risk is summarized in terms of superior diagnostic value, including the *MRI alone* concept, and in terms of patient outcome. Thereafter, the special case of high risk from previous chest radiation therapy (CRT) will be considered.

17.2.2 The Context: Population-Based Screening Programs

Mammography, notwithstanding its intrinsic limitations in terms of sensitivity and specificity, remains the basic tool for population-based mass screening, being demonstrated to be effective in reducing mortality and allowing for conservative therapy [113]. The stage of BC at diagnosis significantly impacts on overall survival even in recent years, when effective systemic therapies are applied. In other words, *early diagnosis remains crucial*. This concept has been recently confirmed by a population-based study from the Netherlands Cancer Registry evaluating more than 170,000 patients: although the rate of those receiving neoadjuvant/adjuvant therapy from 1995–2005 to 2006–2012 increased from 53 to 60%, in 2006–2012, mortality still increased with progress-

[1]Notably, some new currently emerging technologies such as *optical imaging* and *opto-acustic imaging* should not be confused with the old *thermographic* methods. Interesting research on these new approaches is ongoing, and good results may be possible. See, for example, Sella T, Sklair-Levy M, et al. (2013) A novel functional infrared imaging system coupled with multiparametric computerised analysis for risk assessment of breast cancer. Eur Radiol 23:1191–1198.

[2]This comparison was firstly reported in Amsterdam by Pascal Baltzer during the ceremony for the EUSOBI (European Society of Breast Imaging) 2014 Gold Medal to the memory of Prof. Werner A. Kaiser (*05.10.1949, † 27.12.2013).

ing tumor stage, significantly for T1c vs. T1a and independently from nodal status [114].

The International Agency for Research on Cancer (IARC) recently summarized the evidence in favor of screening mammography [59, 115]. The estimated reduction in BC mortality is 40% for those women aged 50–69 who take up the invitation and 23% when also including those not accepting the invitation. A mortality reduction has been also estimated for women aged 40–49 and 70–74, though with "limited evidence" [59]. In addition, we must note that screening mammography allows for both downscaling of the clinicopathological features of invasive BCs and reducing locoregional and adjuvant treatments [51, 116–118].

A good news of the last years is the *end of confusion*, as appropriately stated by the Society of Breast Imaging about harms from screening mammography [119]. This is a hot topic, in particular for *false-positive rate* and *overdiagnosis*. In Europe, the average risk for a false-positive recall is limited to 20% for women aged 50–69 who have ten screens in 20 years; the probability of false-positive needle biopsy is <1% per round [59]. A low rate of overdiagnosis has been calculated by the IARC working group [59], from 1 to 10% or from 4 to 11%, according to different estimation methods. Notably, *overdetection* (a radiological issue) has to be distinguished from *overdiagnosis* (which implies also an essential role of pathologists) [120], while more efforts should be dedicated to the reduction of *overtreatment*.

However, one weak point of current population-based screening programs remains the *one-size-fits-all* rule: in Europe, mammography every 2 years (every 3 years in the United Kingdom) from 49 to 69 years. Some change has been introduced when also women from 40 to 49 (mostly from 45 to 49) are invited: the periodicity is commonly reduced to 1 year only. During the last three decades, organizational issues and other factors worked against the idea to stratify the screening strategy according to the risk level and breast density. The latter factor is relevant: even though density as an independent risk factor is commonly overestimated [121], its masking effect results in a relevant reduction in mammography sensitivity [122]. An organized screening strategy tailored for the woman's individual risk, also considering breast density, is a hope for the future.

Coming to the crucial point, it was clear that the diagnostic performance of mammography in high-risk women was inadequate. The sensitivity ranged 29–50%, the interval cancer rate 35–50%, and the metastatic nodal involvement at diagnosis 20–56% [123]. Something different had to be proposed. A new screening strategy to be implemented had to consider four elements:

1. The need to start very early in the high-risk woman's life, accounting for the early disease onset
2. The need for closer screening events, accounting for the fast BC growth in these women
3. Independence of the screening tool from breast density, accounting for the woman's young age and for the higher breast density in high-risk women
4. Possible avoidance of ionizing radiation exposure, accounting for the higher sensitivity to radiation of BRCA mutation carriers (as explained in detail below)

This was the scenario when the first studies on MRI-including screening programs were initiated. The only change in those years and during the first decade of 2000 was the slow but progressive transition from film-screen to digital mammography, without any substantial impact for high-risk women.

17.2.3 High-Risk Screening with MRI: From a *Mission Impossible* to a Large Body of Evidence

To explore the diagnostic power of CE-MRI in a screening setting was initially a *mission impossible*. The typical objection was the following: *MRI specificity is too low, and you will be flooded by a deluge of false positives*. However, as stated by Thomas Kuhn [124], scientific research is attractive also due to "the excitement of exploring new territory, the hope of finding order, and the drive to test established knowledge".

Thus, different groups started to verify the hypothesis that CE-MRI could be useful for BC screening. For epidemiological reasoning, women at increased BC risk, especially those with hereditary predisposition, were the natural candidates for these projects. A greater expected incidence would have resulted into a higher positive predictive value (PPV) of screening modalities and a smaller sample size needed to evaluate the differences in diagnostic performance among the modalities [125]. This was also a way to begin to dismount, from the side of high risk, the *one-size-fits-all* rule.

In fact, breast radiologists had to get at least a basic information about familial/genetic predisposition to BC [126]:

- Autosomal dominant inherited BCs are only 5% of all cancers (one third of all familial BCs).
- BRCA1/2 mutations explain only about 40% of autosomal dominant inherited BCs (other genes such as TP53, STK11, PTEN, NF1, CHEK2, ATM, BRIP1, and PALP2 explain about 10%), while the remaining 50% has no gene mutation clearly identified. BRCA1/2 deleterious mutations confer a 50–85% LTR.
- Most BCs in very young women are associated with a BRCA1 mutation, a condition which may also show association with ovarian cancer.
- In women carrying a BRCA2 mutation, the risk profile is shifted to a slightly more advanced age, while BCs in males are commonly associated with this type of mutation.

This body of knowledge allows radiologists, who have the possibility to deal with a large number of women on the occasion of screening and diagnostic imaging, to identify those women whose family history indicates the possible presence of an inherited BC predisposition. Software can be used for a preliminary risk evaluation, such as that based on the Tyrer-Cuzick model [127, 128]. Anyway, *radiologists (or other professionals who suspect a BC genetic predisposition) have to refer the woman suspected to be at high risk to a specialized department/center for genetic counseling to define the possibility of genetic testing.* Importantly, in the case of strong family history of BC and/or ovarian cancer without identification of known gene mutations in the family, genetic testing is defined as *inconclusive* and the case is labeled as *BRCAX* [129]. Finally, for different reasons, including unsuitable psycho-oncologic condition, many women with strong family history prefer not to perform any genetic testing.

Thus, since the mid-1990s, the context has been enriched to comprise three basic concepts:

1. Mammography, the only established method for BC screening in general, was not working properly for screening women at high genetic/familial risk.
2. Identification of high-risk populations could be based on clearly established criteria to assess/estimate a BC genetic predisposition.
3. There was a need for several years of clinical experience with CE-MRI of the breast in the diagnostic setting, acquired in academic centers and great hospitals, to participate in suitably designed screening programs.

The first report was published by Christiane K. Kuhl in 2000 [130]. Fifteen cancers were detected in 192 women proven or suspected to be carriers of a BC susceptibility gene. Sensitivity was 33% for mammography, 33% for US, 44% for mammography and US combined, and 100% for CE-MRI; PPV 30%, 12%, and 64%, respectively. A number of studies were followed, and the body of evidence grew up in the last 15 years. When the sample size of high-risk women, the number of screening events, and the number of centers involved increased, the sensitivity of MRI slightly decreased, as expected, but the general trend for a huge difference in diagnostic power, especially in sensitivity, between MRI and the other imaging modalities was confirmed not only in terms of efficacy but also in terms of large-scale effectiveness.

High-risk screening has been the prominent application for breast MRI multicenter studies in the last 15 years, involving 7690 women who performed 18,307 MRI examinations (Table 17.6).

The evidence from prospective studies about MRI including screening protocols was summarized in 2014 [131]. Overall, nine studies [111, 132–139] enrolled more than 5500 women. A total of 392 BCs were diagnosed. Of them, 45% had a diameter ≤ 10 mm (95%; confidence interval [CI], 39–51%), 77% were invasive (95% CI 73–81%), and 52% were G3 invasive (95% CI 46–58%). Of the invasive cases with explorable axilla (not previously treated for BC), 23% had nodal metastatic involvement (95% CI 18–28%). Study-by-study details are reported in Table 17.7.

All these studies contributed to build the body of evidence in favor of the use of CE-MRI for screening women at high BC risk. National and international recommendations and guidelines accepted this indication on the basis of the superior sensitivity of breast MRI, including not only professional and scientific societies such as the American Cancer Society (already mentioned) [109], the American

Table 17.6 Multicenter breast MRI studies from 1997 to 2014

		Patients		MRI exams		Centers		Papers	Journals		Papers per country			
Study type	Studies	Total	Min Max	Total	Min Max	Total	Min Max		Imaging	Other	Europe	Europe and USA	USA	Asia
High-risk screening	10 (24%)	7690	93 2500	18,307	171 7500	157	4 30	29 (43%)	10	19	26	2	1	–
Diagnostic performance and contrast materials	14 (33%)	3989	63 969	5026	63 1652	158	3 25	18 (27%)	15	3	9	4	5	–
MR-guided biopsy/ localization	6 (14%)	2069	132 821	33,386	132 1029	51	3 20	6 (9%)	3	3	5	1	0	–
Preoperative	6 (14%)	2784	90 1623	2030	90 761	76	2 45	7 (10%)	1	6	6	0	0	1
NAT effect evaluation	6 (14%)	1029	89 746	3300	46 746	34	3 15	7 (10%)	0	7	3	0	1	–
Total	42 (100%)	20,348	63 2500	32,049	46 7500	476	2 45	67 (100%)	29 (43%)	38 (57%)	49	7	7	1

USA United States, *NAT* neoadjuvant therapy
Data from PubMed/Medline, accessed on December 22, 2014

College of Radiology [140], the European Society of Breast Imaging [141, 142], or the multidisciplinary European Society of Breast Cancer Specialists (EUSOMA) [143] but also governmental bodies such as the National Comprehensive Cancer Network [144] in the USA and the National Institute for Health and Care Excellence [145] in the United Kingdom.

Differences exist among guidelines, especially for the threshold of LTR to define the indication to MRI, lower (20–25%) in guidelines from the USA and higher (30% or more) in some European guidelines. However, in all guidelines MRI is proposed for screening high-risk women. Key recommendations issued by EUSOMA in 2010 [143] are summarized in Table 17.8.

Table 17.7 Prospective studies on MRI including screening of women with increased familial BC risk

First author year, study name country [Reference]	Subjects enrolled	MRI sensitivity (%)	MRI specificity (%)	MRI-detected invasive cancers ≤10 mm in diameter	Invasive cancers/all cancers	DCIS/all cancers	Invasive grade 3/all invasive cancers	Metastatic nodal involvement/all invasive cancers
Warner 2004, Canada [132]	Mut	77	95	9/16 (56%)	16/22 (73%)	6/22 (27%)	NR	2/15 (13%)
Kuhl, 2005, Germany [133]	Fam/Mut	91	97	NR	34/43 79%)	9/43 (21%)	11/24 (46%)	5/31 (16%)
Leach 2005, MARIBS UK [134]	Fam/Mut	77	81	13/29 (45%)	29/35 (83%)	6/35 (17%)	19/29 (66%)	5/26 (19%)
Hagen 2007, Norway [135]	Mut	86	NR	8/19 (42%)	21/25 (84%)	4/25 (16%)	13/21 (62%)	6/20 (30%)
Riedl 2007, Austria [136]	Fam/Mut	86	92	8/16 (50%)	16/27 (59%)	11/27 (41%)	6/16 (38%)	2/16 (13%)
Rijnsburger 2010, The Netherland [137]	Fam/Mut	71	90	30/74 (40%)	78/97 (80%)	19/97 (20%)	28/72 (39%)	22/72 (31%)
Kuhl 2010, EVA Germany [138]	Fam/Mut	93	98	9/16 (56%)	16/27 (59%)	11/27 (41%)	6/16 (38%)	NR
Sardanelli 2011, HIBCRIT, Italy [111]	Fam/Mut	91	97	15/39 (38%)	44/52 (85%)	8/52 (15%)	28/44 (64%)	11/39 (28%)
Evans 2014, MARIBS UK [139]	Fam/Mut	93-100	63	24/47 (51%)	47/63 (75%)	16/63 (25%)	29/47 (61%)	8/47 (17%)

MRI magnetic resonance imaging, *DCIS* ductal carcinoma in situ, *Fam* women at elevated familial risk of breast cancer, *Mut* women proven to carry a deleterious mutation in a breast cancer susceptibility gene (*M*), *M* mutation carriers only, *NR* not reported (From Santoro et al. 2014 [131], modified, with permission)

Table 17.8 Ten key points on screening women with an increased BC risk from EUSOMA recommendations

1.	Women with a family history suspicious for inherited BC predisposition should have their risk assessed by an appropriately trained professional group (genetic counseling); LTR thresholds for including women in surveillance programs with annual MRI may be selected on the basis of regional or national considerations
2.	High-risk screening including MRI should be conducted only at a nationally/regionally approved and audited service or as part of an ethically approved research study. Periodical audit should be undertaken to ensure that high sensitivity is achieved and recall rate (MRI more frequently than annual) is less than 10% and to monitor detection rate, needle biopsy rate, and interval cancers
3.	Annual MRI screening should be available starting from the age of 30. Starting screening before 30 may be possible for BRCA1/2 mutation carriers (from 25 to 29) and TP53 (from 20)
4.	Annual MRI screening should be offered to BRCA1, BRCA2, and TP53 mutation carriers; women at 50% risk for BRCA1, BRCA2, or TP53 mutation in their family (first-degree relatives of mutation carriers); and women from families not tested or inconclusively tested for BRCA mutation with a 20–30% LTR or greater
5.	MRI including screening should be offered also to high-risk women previously treated for BC
6.	Screening mammography should not be performed in high-risk women below 35. In TP53 mutation carriers of any age annual mammography can be avoided based on discussion on risks and benefits from radiation exposure
7.	Annual mammography may be considered for high-risk women from age 35
8.	If annual MRI is performed, screening the whole breast using US and clinical breast examination are not necessary. They are recommended in women under 35 who do not tolerate or have contraindication to MRI or to Gd-based contrast material administration
9.	Cases requiring workup after MRI should be initially assessed with conventional imaging (reevaluation of mammograms, targeted US). In case of only MRI-detected suspicious findings, MR-guided biopsy/localization should be performed
10.	Risk factors such as heterogeneously or extremely dense breasts, previous diagnosis of breast invasive cancer or ductal carcinoma in situ, atypical ductal hyperplasia, and lobular intraepithelial neoplasia, when not associated with other risk factors, do not confer an increased risk that justifies MRI screening

BC breast cancer, *LTR* lifetime risk, *MRI* contrast-enhanced magnetic resonance imaging, *US* ultrasound. From Sardanelli et al. [142], modified. Notably, the EUSOMA recommendations include also women who underwent chest radiation therapy, here discussed in the section 17.2.6.

Secondary evidence in terms of systematic reviews were published, generally confirming the introduction of annual CE-MRI for high-risk screening in terms of both diagnostic performance [146–148] and cost-effectiveness [149].

One relevant contribution came from an individual patient data meta-analysis [150], authored by a team including authors of six original studies. It was demonstrated that the addition of MRI to mammography for screening BRCA1/2 mutation carriers aged ≥50 improves screening sensitivity by a similar magnitude to that observed in younger women. This means that those guidelines which limit screening MRI in BRCA1/2 mutation carriers only up to 50 years of age should be updated to this new evidence.

17.2.4 Radioprotection Issues and the *MRI Alone* Approach

The idea of avoiding mammography in carriers of gene mutations conferring an increased BC risk is not new. It was related to the well-known role of oncosuppressor genes such as BRCA1 and BRCA2. Studies on animal model had shown that BRCA2 protein interacts with the DNA repair protein Rad51, explaining a higher radiation sensitivity [151]. Thus, also from our side [152], we suggested the possibility to abstain from doing mammography at least up to age 35, taking into consideration that, on the basis of available studies, the rate of undetected BCs was only 4%, limited to only ductal carcinoma in situ (DCIS).

This view was subsequently confirmed by statistical modeling of the risk of radiation-induced BC from mammographic screening for young BRCA mutation carriers [153] and by the empiric demonstration of more DNA double-strand breaks induced by mammographic exposure in human mammary epithelial cells sampled from patients with high than with low family BC risk, with a dose-effect exacerbated in cells from high-risk women [154].

Moreover, mammography could be avoided also from the viewpoint of a limited diagnostic performance. This was very clear especially after the results of the EVA study conducted in Germany [138] and of the HIBCRIT study conducted in Italy [111]. The EVA study, based in four academic institutions, included 687 asymptomatic women with familial high risk (LTR ≥20%) who underwent 1679 annual screening rounds composed by clinical breast examination (CBE), mammography, US, and MRI; in a subgroup of 371 women, additional half-yearly ultrasound and CBE were performed in more than 869 rounds. Of 27 BCs diagnosed (11 DCIS and 16 invasive), 3 (11%) were node positive. After a mean follow-up of 29 months, no interval cancers occurred; no cancer was identified by half-yearly ultrasound examinations. No significant difference in detection rate was observed between US (6.0%) and mammography (5.4%), with a not significant increase to 7.7% for both modalities combined. MRI alone had a significantly higher detection rate (14.9%), unchanged by adding US and not significantly increased by adding mammography (MRI plus mammography, 16.0%) , and not changed by adding ultrasound (MRI plus ultrasound, 14.9%). The PPV was 39% for mammography, 36% for US, and 48% for MRI.

Similar results were obtained by the HIBCRIT study [111], based in 18 cancer centers, universities, and general hospitals. We enrolled 501 asymptomatic women aged ≥25 who were BRCA mutation carriers, who were first-degree relatives of BRCA mutation carriers, or women with strong family history of BC or ovarian cancer, including those with previous personal BC. A total of 1,592 rounds were performed; 49 screen-detected and 3 interval cancers were diagnosed: 44 invasive and 8 DCIS; and 4 being pT2 stage, 32 G3 grade. Of 39 patients explored for nodal status, 28 (72%) were negative. Incidence per year-woman resulted significantly higher at ≥50 years of age (5.4%) than at <50 years of age (2.1%), 3.3% overall, significantly higher (4.3%) in women with previous personal BC than in those without (2.5%). The diagnostic performance of CBE, mammography, US, and their combinations is reported in Table 17.9.

At receiver-operating characteristic analysis, MRI showed a superior diagnostic performance than mammography or US (0.82), while MRI combined with mammography and/or US did not overrun MRI alone (Fig. 17.1). Of 52 cancers, 16 (31%) were diagnosed only by MRI. An example of the superior sensitivity of MRI is shown in Fig. 17.2.

Both the German and the Italian studies showed that MRI largely outperforms mammography, US, and their combination. While the EVA trial added the relevant information that US, even when performed every 6 months, does not add sensitivity, the HIBCRIT study demonstrated the effectiveness of an MRI including screening protocol on the large scale of 18 centers. The PPV values were about 50 and 60% for the two studies, respectively, a certainly good metrics in a screening setting. Of note, specificity of MRI was obviously very high in both studies, as of course expected when the probability of the true negative is overwhelming. However, only very recently the *mantra* about the low specificity of breast MRI has begun to reduce its credibility.

The key point of the superior sensitivity of MRI is due to the high detection of small cancers. In the HIBCRIT study, the sensitivity for pT1a–b BCs was 10/20 (50%) for mammography plus US vs. 95% for MRI. Moreover, in an explorative analysis, we also showed no gain in sensitivity as an effect of the transition from film-screen (17/31, 55%) to digital mammography (8/19, 42%) [112].

This new *MRI alone* paradigm, i.e., the absence of additional diagnostic power by adding other imaging modalities after a negative MRI, is due to the very high sensitivity and specificity of the method. For statistical reasons, it is quite

Table 17.9 Diagnostic performance of the different modalities in the HIBCRIT study

Modality	Sensitivity (%)	Specificity (%)	PPV2 (%)	NPV (%)	LR+	LR−
Clinical breast examination	17.6	99.4	60.0	96.1	30.9	0.83
Mammography	50.0	99.1	73.5	97.6	58.1	0.50
Ultrasound	52.0	99.2	76.5	97.7	66.0	0.48
MRI	91.3*	97.4	61.8	99.6*	35.1	0.09*
Mammography + ultrasound	62.5	98.4	65.2	98.2	39.0	0.38
MRI + mammography	93.2	97.0	58.6	99.7	31.5	0.07
MRI + ultrasound	93.3	97.1	60.0	99.7	32.0	0.07

PPV2 positive predictive value 2 (needle biopsy prompted), *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−* negative likelihood ratio, *MRI* contrast-enhanced magnetic resonance imaging. * indicates that the MRI value is signficantly better than each of the the other modality or their combinations.

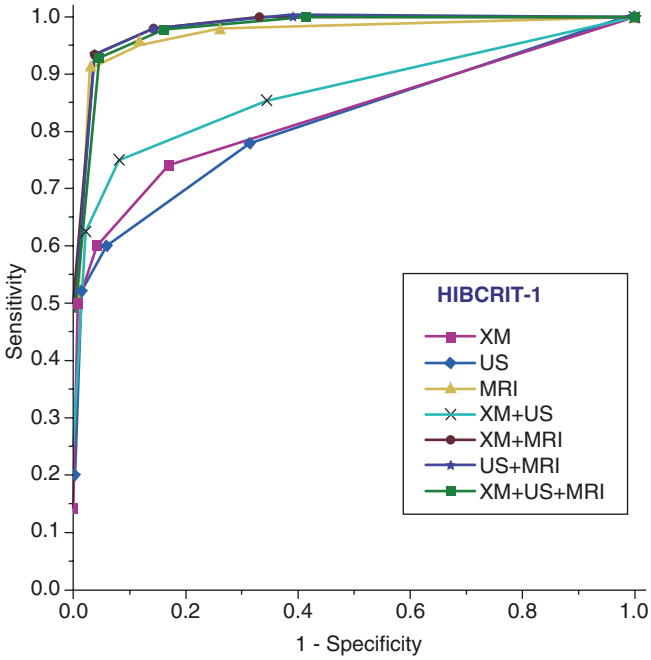

Fig. 17.1 Receiver-operating characteristic analysis of diagnostic performance of mammography (XM), ultrasound (US), MRI, and their combination for screening high-risk women. The AUC of MRI (0.97) was significantly higher than that of mammography (0.83) or US (0.82) and not significantly increased when MRI was combined with mammography and/or US. HIBCRIT study [111]

unlikely that any other technique can add significant diagnostic gain, unless a huge sample size is considered.

This approach has been reinforced by the results of a number of subsequent studies. A study from Ontario, Canada, reported on the initial evaluation of 2207 high-risk women [156]: of 35 BCs detected, none was identified by mammography alone. A study from the Netherlands considering only BRCA1 mutation carriers [157] reported on 82 invasive BCs and 12 DCIS during the study. They had four interval cancers (all invasive): MRI missed only 2 DCIS that were detected by mammography (2/94, 2%). An update from the Austrian study [158] showed that of 40 BCs 18 (45%) were detected by MRI alone and only two by mammography alone (a DCIS with microinvasion and a DCIS with <10 mm invasive areas), without leading to a significant increase in sensitivity vs. MRI alone; no cancers were detected by US alone.

Finally, an individual patient data meta-analysis including six high-risk studies [159] recently showed that in BRCA1/2 mutation carriers, adding mammography to MRI did not significantly increase sensitivity. However, the increase was 3.9% in BRCA1 but reached 12.6% in BRCA2 mutation carriers. In women with BRCA2 mutation younger than 40 years, one third of BCs were detected by mammography only. We should consider here that the inclusion of only six studies, based on the voluntary contribution of the individual patient data by the authors of the original researches, did not allow for including data from some other studies which could have reduced the rate of BCs detected on mammography only.

At any rate, due to the very low, if any, contribution of US and the low contribution of mammography when compared to MRI for screening a high-risk population, we can propose the following simple recommendations:

1. MRI alone up to 35 years of age for all high-risk women
2. MRI alone for BRCA1 and p53 mutation carriers without age limitations
3. *Mammography as an adjunct to MRI* for BRCA2 mutation carriers after 35 years of age

Thus, the paradigm *MRI as an adjunct to mammography* has been reverted into its contrary. When *mammography as an adjunct to MRI* is under consideration for high-risk women, a good conservative approach has been suggested, consisting of performing only one projection, the mediolateral oblique one [160].

17.2.5 Impact on Patient Outcome

If the principles of evidence-based medicine [161] are applied to screening programs, a high detection rate or a very good diagnostic performance of a screening tool should not

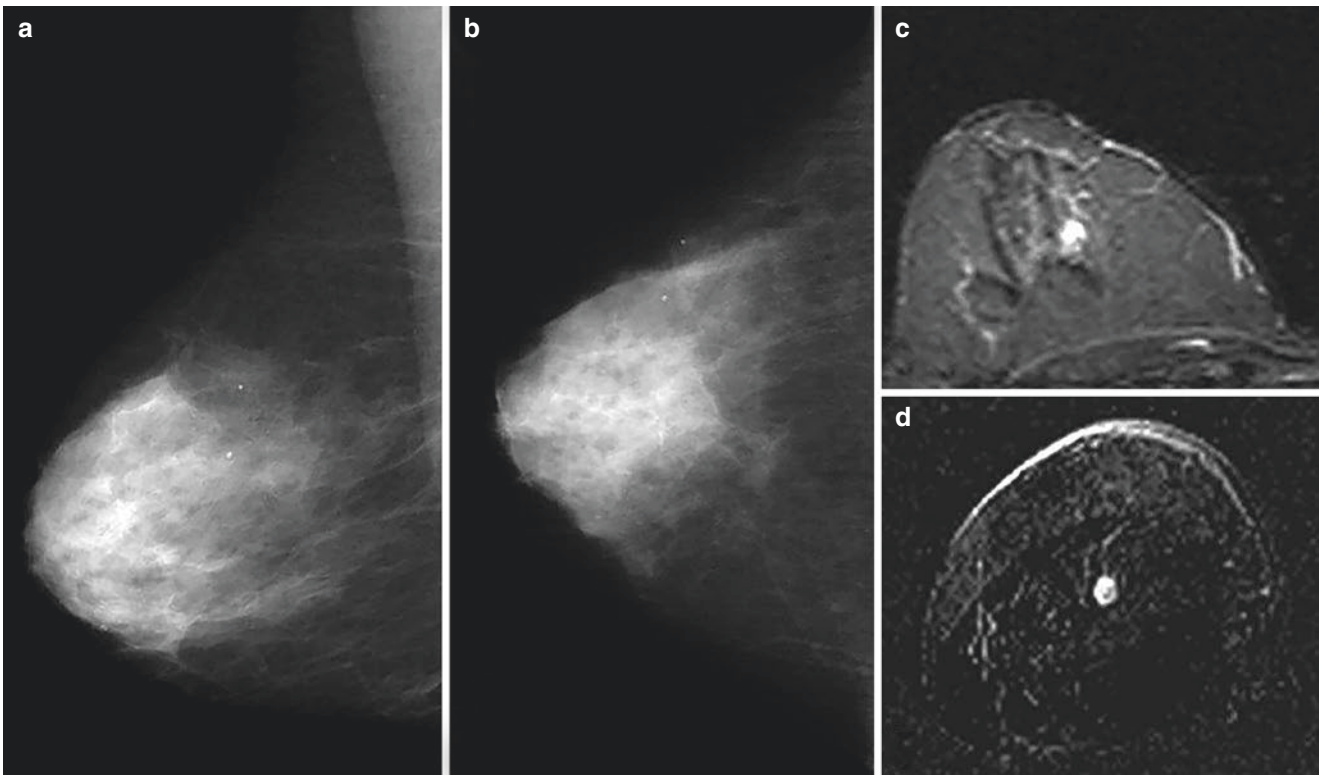

Fig. 17.2 Case from the HIBCRIT study. A 53-year-old BRCA1 mutation carrier, already treated for an invasive ductal cancer of the left breast at 33 years of age, underwent multimodal screening including clinical breast examination (CBE), mammography, US, and MRI. The left breast only showed minimal signs of the previous treatment at each screening modality (not shown). Mammography of the right breast showed a negative dense breast (**a**) and (**b**). Also CBE and US (not shown) were negative; at MRI the unenhanced T2-weighted axial short-tau inversion-recovery sequence (**c**) showed a small hyperintense mass, confirmed at the subtracted (contrast-enhanced minus unenhanced T1-weighted gradient echo) coronal image (**d**). Final diagnosis: node-negative invasive ductal carcinoma (6 mm in diameter) (From Podo et al. 2016 [155], with permission)

be considered *per se* as a sufficient reason to implement this screening tool in practice. Randomized controlled trials should be performed to take into account lead time bias, length bias, and overdiagnosis, finally evaluating whether the screening under consideration has a significant impact on mortality and patient outcome overall.

This rule should be theoretically also applied to high-risk population. However, ethical issues make this approach (i.e., to obtain information from randomized controlled trials) no longer possible for what we are considering in this chapter. The demonstrated gap in sensitivity between MRI and mammography and/or US is too high to propose a randomization to a BRCA or p53 mutation carrier. We are convinced that no ethics committee would approve such a protocol.

Therefore, we had to refer to an indirect evidence. On the one side, an impact of the anticipated diagnosis obtained with MRI in a high-risk population can be inferred considering the impact of screening mammography on the general female population [114]. On the other side, relevant information began to come from the cohorts included in the above-mentioned high-risk studies.

Rijnsburger et al. [137] reported a 5-year cumulative overall survival higher in the prospective MRI screening patient series of the Dutch MRISC study (93%) than in institutional historical unselected controls, as well as in 26 published series. This result was associated with a more favorable tumor stage, particularly in a moderate-risk group.

Møller et al. [162] reported on survival of patients with BRCA1-associated BCs diagnosed in an MRI-including screening program. The 5-year BC-specific survival for women with cancer was 75%, and the 10-year survival was 69%. The 5-year survival for women with stage 1 BC was 82% compared to 98% in the general population. The authors commented that these survival rates were *less than anticipated* and *the benefit of annual MRI surveillance on reducing BC mortality in BRCA1 mutation carriers remains to be proven.*

We argue that one key point is the historical context of the cohorts of screened women, i.e., the associated effect of early diagnosis combined with that of modern treatment protocols to better exploit the advantage of an early MRI detection. When Evans et al. [139] compared three cohorts

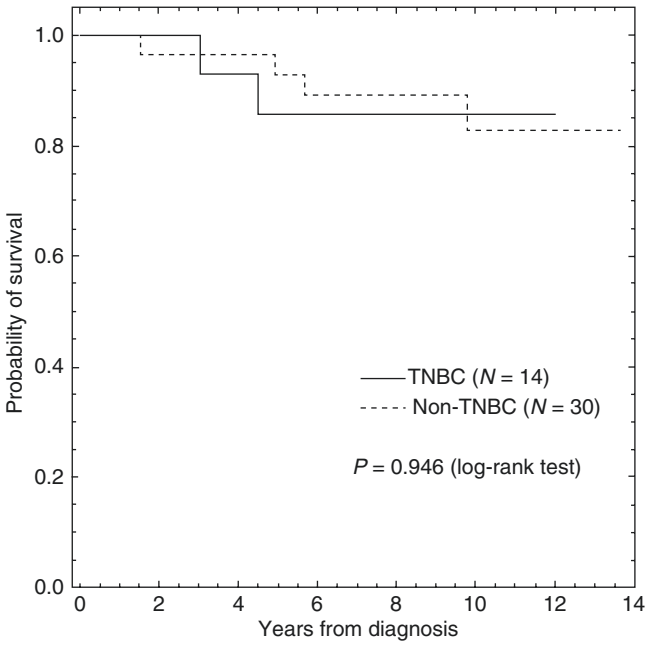

Fig. 17.3 Kaplan-Mayer analysis of overall survival of high-risk women affected with triple-negative breast cancers (TNBC) or non-TNBC during the HIBCRIT study [111] (with permission)

of high-risk women who had no screening, mammography or an MRI including program, a clear advantage of mammography vs. no screening and MRI vs. mammography or no screening is visible. However, these three cohorts are not concurrent, but subsequent and their survival should have been influenced by the progressive improvement of therapies [131].

Our contribution has been to compare phenotype features and survival of triple-negative BCs (TNBCs) vs. non-TNBCs detected during the HIBCRIT study [155], on the basis of a median of 9.7-year follow-up. The 44 invasive BCs (41 screen-detected and 3 BRCA1-associated interval TNBCs) comprised 14 TNBCs (32%) and 30 non-TNBCs (68%), without significant differences for age at diagnosis, menopausal status, prophylactic oophorectomy, or previous BC. Of 14 TNBC patients, 11 (79%) were BRCA1; of the 20 BRCA1 patients, 11 (55%) had TNBC; and of 15 patients enrolled for family history only, 14 (93%) had non-TNBCs. TNBC patients had more frequent ipsilateral mastectomy, contralateral prophylactic mastectomy, and adjuvant therapy. The 5-year overall survival was 86% ± 9% for TNBCs vs. 93% ± 5% for non-TNBCs; 5-year disease-free survival was 77% ± 12% vs. 76% ± 8%, respectively, without significant differences (Fig. 17.3). We are aware that the detection of TNBCs in BRCA (especially BRCA1) mutation carriers could have been responsible for the selection of more drastic therapies vs. those decided for noncarriers, so that the relative contribution of MRI and systemic therapies is not easily discernible [163]. At any rate, the relevant clinical message here is that, in high-risk women, by combining an MRI-including annual screening with adequate treatment, the usual reported gap in outcome between TNBCs and non-TNBCs could be reduced.

17.2.6 The Special Case of Previous Chest Radiation Therapy

Women who underwent chest radiation therapy (CRT) during pediatric/young-adult age (typically those treated for Hodgkin's lymphoma) have an increased BC risk, in particular those who received mantle CRT with high doses. The cumulative BC incidence from 40 to 45 years of age in these women is 13–20%, higher than that observed in the young female general population and similar to that of BRCA mutation carriers. The risk is higher for high doses delivered between 10 and 16 years of age. The BC is diagnosed on average about 15 years after CRT at about 40, to be compared with a mean age of about 61 in the general female non-exposed population [164, 165]. These BCs are similar to those encountered in the general female population in regard to histopathologic subtype, receptor status, lymphatic invasion, and nodal involvement. Of note, BCs in women who underwent CRT exhibit a preferential localization at upper external quadrants more extreme than that observed in women with hereditary predisposition (67% vs. 48%, respectively); moreover, in these women the possibilities of treatment of BC mostly exclude radiation therapy and chemotherapy with doxorubicin [166].

For women who underwent CRT, guidelines [109, 143, 167] recommend annual mammography and CE-MRI, starting from 25 years of age or, for those women who had CRT before 30, 8 years after the end of treatment. The rationale is the similar BC incidence in the young age for women who had CRT and women with hereditary predisposition associated with relatively lower sensitivity of mammography, also related to the need to start at a young age, and higher sensitivity of MRI.

In the USA, a study published in 2009 [168] reported that, of 551 women with previous CRT, 47% of those with 25–39 years of age never had a mammogram and only 37% had biannual screening mammography, the same percentages being 8 and 53% between 40 and 50 years of age. Importantly, the screening rate was higher in the presence of a specific medical recommendation.

Before the MRI introduction, the breast surveillance of women with previous CRT included annual physical examination and mammography [169]. This protocol allowed for detecting 60% of BC in the preinvasive phase or at T1 stage [170–174]. Two prospective [175, 176] and two retrospective studies [177, 178] compared mammography and MRI. Sensitivity ranged from 67 to 70% for mammogra-

phy, from 63 to 80% for MRI, with a 92% sensitivity reached only in one retrospective study, with a very small sample size for MRI [178]. Importantly, in women who underwent CRT, MRI sensitivity is relatively lower (63–80%) and that of mammography is relatively higher (67–70%) than those observed in women with hereditary predisposition, due to a higher incidence of DCIS with microcalcifications [179] and low neoangiogenesis. A sensitivity close to 95% can be obtained only using mammography as an adjunct to MRI.

An expert panel [180] recently compared the recommendations proposed by the following working groups: North American Children's Oncology Group (COG), Dutch Childhood Oncology Group (DCOG), Scottish Intercollegiate Guidelines Network (SIGN), and UK Children's Cancer and Leukaemia Group (UKCCLG). As a result of this comparison, a series of "harmonized recommendations" were provided: physicians, health-care providers, and women who had CRT should be informed on the treatment-related BC risk (strong recommendation); the surveillance is recommended for doses ≥20 Gy (strong recommendation); the surveillance is reasonable for doses between 10 and 19 Gy, taking into account the clinical context and further risk factors (moderate recommendation); the surveillance may be reasonable for doses between 1 and 9 Gy, taking into account the clinical context and further risk factors (weak recommendation); the surveillance implies annual check from 25 years of age or, at least, 8 years after CRT up to 50 years of age using mammography, MRI, or both of them (strong recommendation); and physical examination may be reasonable in countries where only clinical surveillance is available (weak recommendation).

Considering the available evidence, women who underwent CRT before 30 receiving a cumulative dose ≥10 Gy should be invited after 25 (or, at least, 8 years after CRT) to attend the following program [181]:

1. Dedicated interview about individual risk profile in order to define the potential of different breast imaging modalities in this specific setting
2. Annual CE-MRI using the same protocol recommended for women with hereditary predisposition
3. Annual bilateral two-view full-field digital mammography or digital breast tomosynthesis (DBT) with synthetic two-dimensional reconstructions

When reaching the age for entering population screening program, the individual risk profile should be discussed with the woman to opt for the only mammography/DBT screening or for continuing the intensive protocol including MRI.

Conclusions

More than 20 years after the identification of BRCA gene mutations and 20 years after the introduction of CE-MRI, evidence has been accumulated in favor of MRI-including screening programs for high-risk women. In some conditions, especially for BRCA1 mutation carriers, *MRI alone* can be proposed. Importantly, in the case of previous CRT, *mammography as an adjunct to MRI* is always recommended as a high incidence of DCIS with microcalcifications and low neoangiogenesis limits MRI sensitivity.

The challenge for public health programs is to integrate these protocols for high-risk women into the general screening organization as models for a future stratification of BC screening protocols on the basis of different risk classes, up until a modulation based on the individual risk estimate will be possible, including a possible reduction of screening invitation to very low-risk women.

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Innovation in Breast Cancer Radiology

18

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18.1 Mammography and Tomosynthesis

Rumana Rahim and Michael J. Michell

Abstract X-ray mammography remains the most effective technique for routine breast cancer screening and plays a key role in imaging the breast in symptomatic patients aged from 40 years. The quality of mammography images has improved significantly during the last decade with the introduction of full-field digital mammography. However, although it is the most acceptable and effective technique used for population-based screening with reported breast cancer mortality reductions of up to 20%, the sensitivity and specificity of mammography are limited particularly in younger women and in those with dense glandular breasts.

In modern practice, mammography is complimented by other imaging modalities to improve lesion detectability and characterisation and establish disease extent. These techniques include ultrasound and contrast-enhanced MRI as well as newer mammographic techniques including digital breast tomosynthesis and contrast mammography.

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In this chapter, we discuss the strengths and limitations of mammography in the diagnosis of breast disease and the role of advanced techniques in both diagnosis and screening practice.

18.1.1 Mammographic Technique

Full-field digital mammography is now the standard of care, and the optimisation of technical factors including automatic exposure control, contrast to noise ratio, detail detection, and radiation dose is required for the detection of subtle signs seen with early breast cancer. Breast composition can vary significantly between normal individuals with differing proportions of fat, glandular, and stromal tissue. The correct equipment and technique should allow for the wide variation of patient build, breast size, as well as variations in anatomy.

Careful and skilled positioning of the breast including keeping the patient at ease is essential. Firm and even compression of the breast improves contrast by reducing radiation scatter, improves resolution by reducing tissue overlap; allows for reduced dose, uniform density and minimises both geometric and movement unsharpness. This is essential for the detection of both fine microcalcification and subtle soft tissue signs, for example, distortion.

Standard mammography includes two views of each breast. These are the medio-lateral oblique view and the craniocaudal view (Fig. 18.1). The medio-lateral oblique (MLO) view is performed by angulating the X-ray tube between 30 and 60° depending on patient build, often 45°. The nipple should be in profile, and the anterior surface of the pectoralis major should be visible to the level of the nipple. The inframammary skinfold should be visible with no superimposed skinfolds on the breast. This projection demonstrates more breast tissue than any other projection. The aim of this view is the complete visualisation of breast parenchyma and the retromammary fat as well as low axillary nodes. Review of the area anterior to the pectoralis

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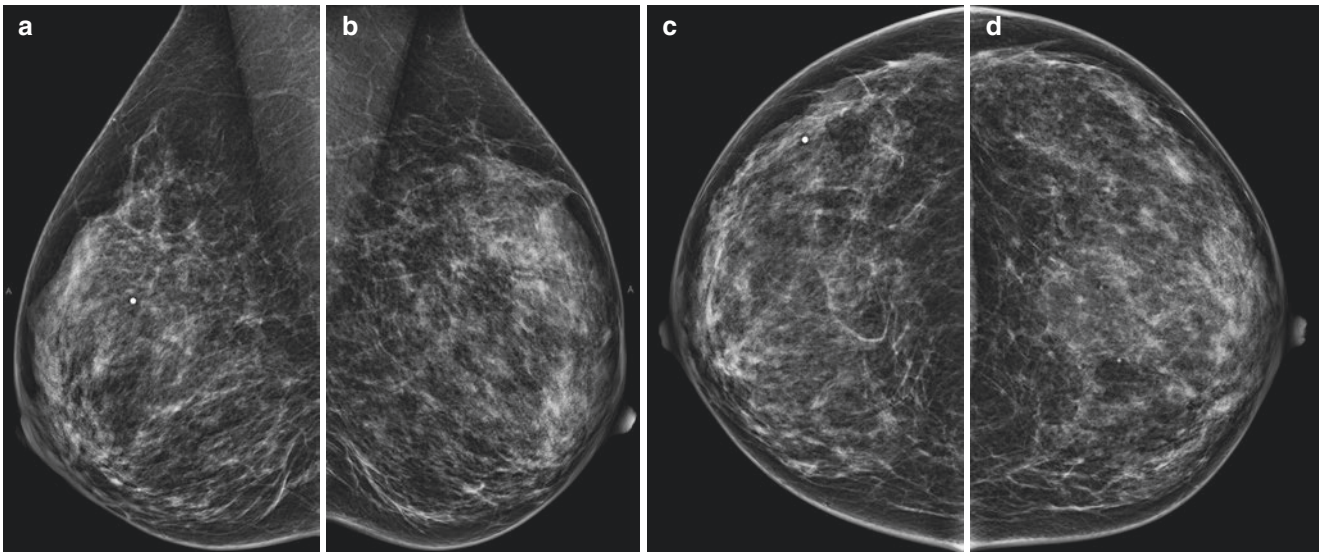


Fig. 18.1 (**a**–**d**) Normal two-view mammograms. (**a**) RMLO, (**b**) LMLO, (**c**) RCC, (**d**) LCC

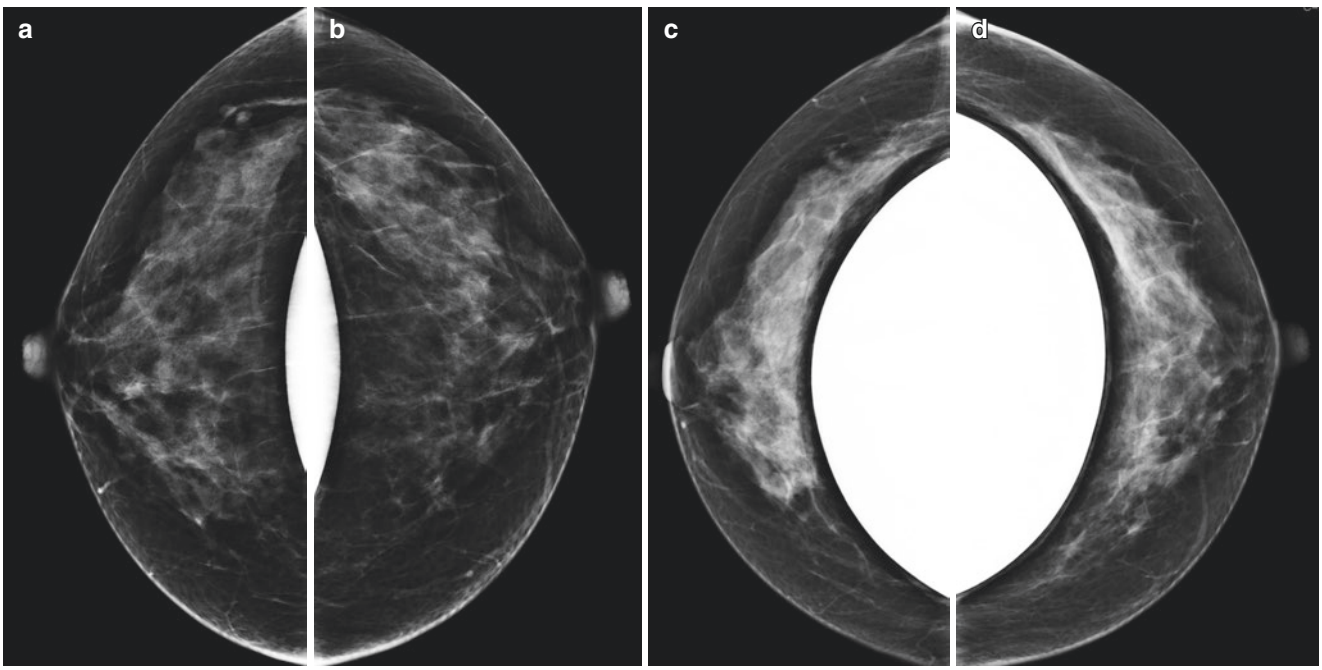


Fig. 18.2 (**a**) and (**b**) are Eklund projections which improve the visualisation of breast tissue in women with implants. (**c**) and (**d**) are standard cc projection mammograms in a woman with implants

muscle and in the immediate retroareolar region is important for the detection of small breast cancers. An important area of the breast not consistently well demonstrated on the MLO projection is the upper inner quadrant which is better demonstrated on craniocaudal (CC) view. The craniocaudal view is performed with a vertical X-ray beam. The nipple again should be in profile with as much breast parenchyma as possible visualised, particularly that in the retroareolar, medial and lateral aspects of the breast as well as the retromammary fat.

In addition to the standard views, supplementary views can be used to improve the visualisation of abnormalities or areas of the breast. Medially or laterally extended craniocaudal views can be performed by rotation of the patient. Fine-focus magnification views increase resolution and are used for the improved visualisation and characterisation of microcalcification. Focal compression views can be used to improve the characterisation of soft tissue densities, asymmetrical densities as well as distortions by displacing the overlying tissues. The Eklund technique is a modified compression view for patients with breast augmentation (Fig. 18.2). The implant is displaced toward the chest wall with anterior traction of the breast tissue to improve visualisation.

18.1.2 Anatomy

The female breast typically has 12–15 lobes which contain terminal ductal lobular units (TDLU). The TDLU consists of acini and lobules which are constantly changing in size and number according to hormonal status. The lobes are variable in size, and the anatomical boundaries of these lobes are not restricted by the quadrants often used to describe disease location in radiology. The shape and contour of the breast are also influenced by supportive fascia and Cooper's ligaments. The wide spectrum of appearances seen on a normal mammogram is reflective of the heterogeneity in breast composition. The parenchymal subtypes were described in the original classification system by Wolfe [1] to enable the recognition of normal mammographic structures.

Breast density is an important descriptor in the interpretation of mammograms. It has significant effects, both on sensitivity and specificity. The mammographically dense breast is an independent risk factor for breast cancer. Breast density is determined by genetic factors and is influenced by age, weight, pregnancy/lactation, medication including exogenous hormones (inhibitors) and breast disease, for example, inflammation. Breast density can be assessed subjectively by the radiologist, but quantitative systems, for example, Volpara and Qantra, provide more accurate, reproducible data. The most widely used classification system in current practice is the BI-RADS (Breast Imaging-Reporting and Data System).

BI-RADS 1: The breast(s) is almost entirely adipose with <25% glandular tissue (Fig. 18.3).

BI-RADS 2: The breast(s) has scattered fibro-glandular tissue occupying 25–50% of the breast (Fig. 18.4).

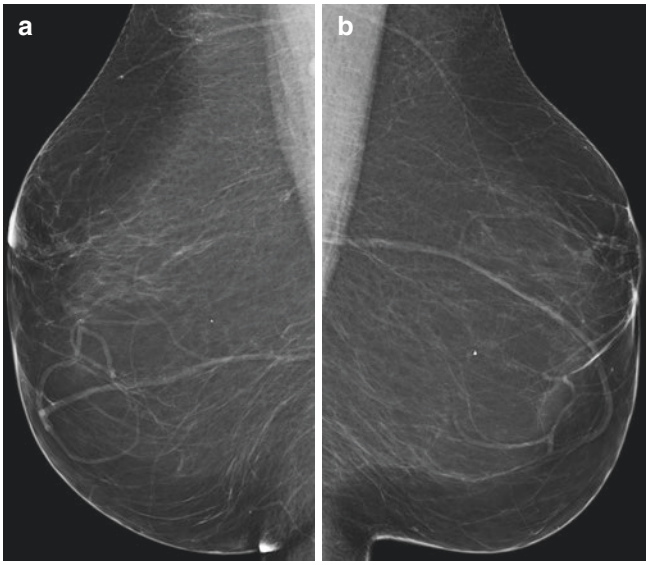


Fig. 18.3 (**a–b**) MLO projection mammograms demonstrate the BI-RADS classification of mammographic breast density. BI-RADS 1

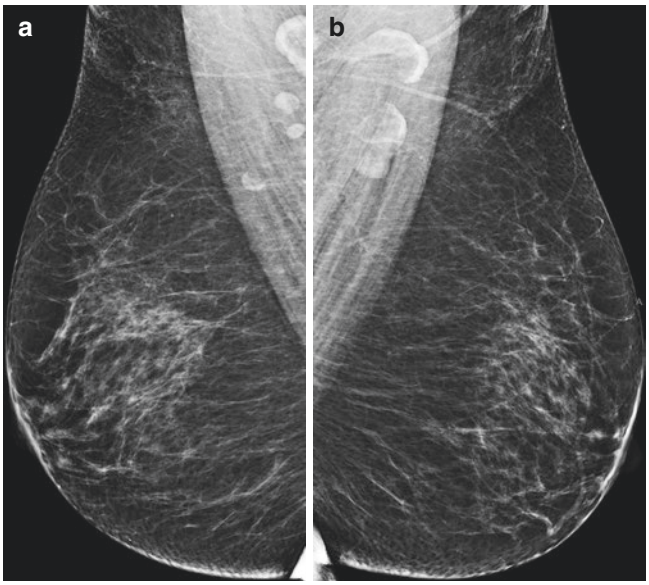


Fig. 18.4 (**a–b**) MLO projection mammograms demonstrate the BI-RADS classification of mammographic breast density. BI-RADS 2

BI-RADS 3: The breast(s) has heterogeneously dense fibro-glandular tissue ranging between 50 and 75% (Fig. 18.5).

BI-RADS 4: The breast(s) has extremely dense 75–100% glandular tissue (Fig. 18.6).

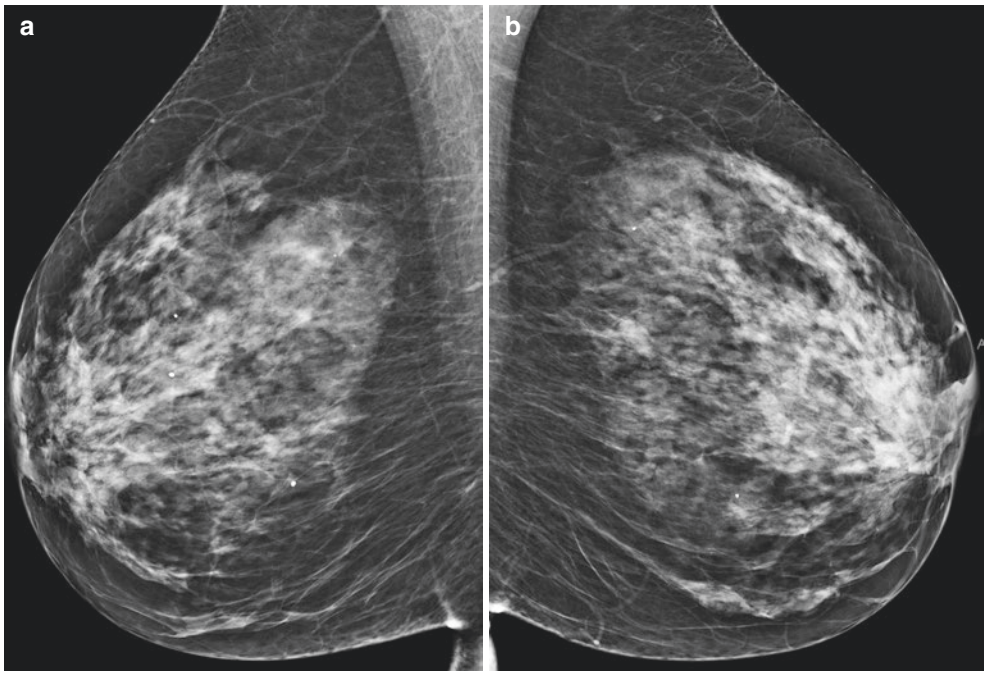


Fig. 18.5 (**a–b**) MLO projection mammograms demonstrate the BI-RADS classification of mammographic breast density. BI-RADS 3

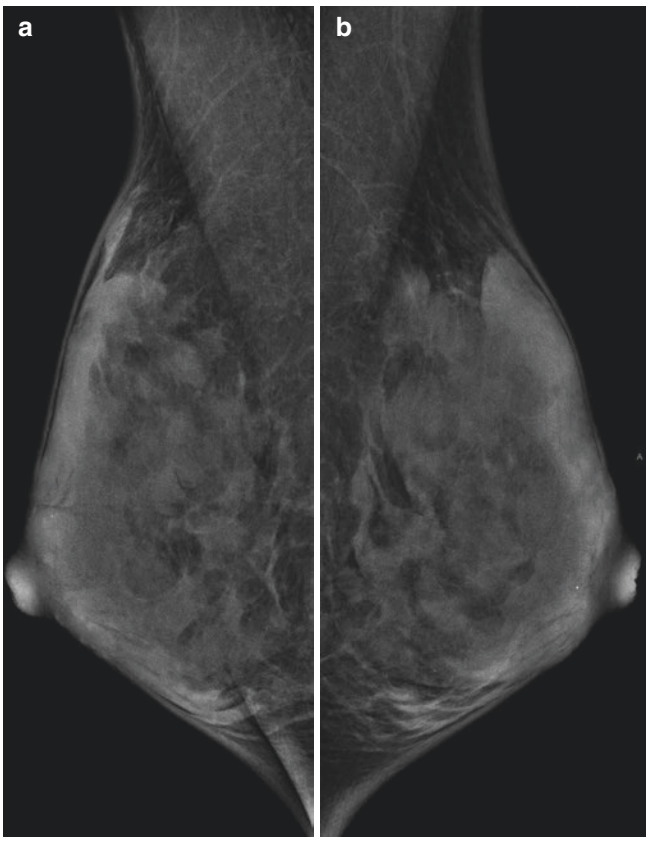


Fig. 18.6 (**a–b**) MLO projection mammograms demonstrate the BI-RADS classification of mammographic breast density. BI-RADS 4

18.1.3 Indications

18.1.3.1 Symptomatic Patients

The triple assessment approach to patients in diagnostic clinics includes clinical breast examination, imaging and biopsy, if required. This ensures an efficient diagnostic process which results in a definitive diagnosis of normal physiological change, benign or malignant disease. Mammography is routinely used in symptomatic patients who present to the breast clinic and are aged 40 years or over. Mammography should also be considered in those below 40 years presenting with signs and symptoms suspicious for breast cancer and in the investigation of male patients over 50 years with a unilateral firm subareolar mass. Standard two-view mammography in symptomatic patients may be complimented with supplementary mammographic views or digital breast tomosynthesis for further characterisation of mammographic features. Ultrasound should be carried out for any patients with breast lumps, persistent focal lumpiness, change in breast size with signs of oedema, change in breast contour, skin tethering or skin dimpling and women with implant-related symptoms as described by Willet et al. [2].

18.1.3.2 Population Screening

Screening mammography is the modality of choice for routine population screening and has been shown to reduce the mortality rate in those invited to breast screening by approximately 15–20%. A meta-analysis of screening trials by Tabar et al. [3]

demonstrated an overall reduction in breast cancer mortality of 22% with an invitation to breast cancer screening. The paper showed a strong relationship between decreased rates of advanced stage (stage II and higher) tumours and lower breast cancer-specific mortality. The mortality benefit from mammographic screening continues following screening as shown on follow-up data from the Swedish randomised control trials published by Tabar et al. [4].

A screening programme must be underpinned by robust quality assurance and measurements of performance to ensure high quality. Mammographic screening should include MLO and CC projections of each breast. The most common age range for routine screening is 50–70 years. The UK age extension trial is a multicentre trial examining the effect of screening in women aged 47–50 and 70–73 and is to report in 2022–2026. Over 2 million women have been recruited with over 200,000 screens to date. Moser et al. [5] reported the age extension pilot results which showed a 0.5% cancer detection rate in those 47–49 years and 1.1% in those 71–73 years with recall rates 2.5 times higher in those 47–49 years compared with the 71–73 years group. The US Preventive Services Task Force published recommendations in 2009 [6] of biennial screening for women 50–74 years for general population breast screening and no screening for those aged 40–49 years except on an individual case basis. The effectiveness of mammographic screening of women aged 40–49 is less certain than for those over 50 years.

The frequency of mammography is variable dependent on individual risk and the screening programme. Intervals vary from 1 to 3 years. The UK programme invites women triannually. However, Dibden et al. [7] demonstrated 44% of interval cancers in this programme to be in the third year following negative screen, suggesting that 3 years is too long an interval for screening. Biennial screening has been suggested as optimal for normal population screening.

Women of greater than average population risk of breast cancer can be categorised as moderate or high risk. The UK categorises women at high risk if they have a lifetime risk of 30% and moderate risk between 17 and 29%. The European Society of Breast Cancer Specialists recommends high-risk screening for women with a lifetime risk greater than 20–30%, and the American College of Radiology recommends high-risk screening for those with a lifetime risk of >20%. Moderate risk is classified in Europe and the USA as a lifetime risk of >15%. There are a number of risk calculation tools including the Claus model, Gail model and BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm). The BRCAPRO and Manchester scoring system are used specifically for the assessment of BRCA mutations.

Mammographic screening is the mainstay in women with a moderate lifetime risk with annual mammograms. Moderate-risk women may have a family history without known genetic mutations. Other risk factors include women with a personal history of breast cancer; benign conditions like atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), and lobular carcinoma in situ (LCIS); and dense breasts. Women with a previous personal diagnosis of breast cancer have a 5–10% risk of recurrence in the first 10 years. The mammographic sensitivity is compromised in women who have had breast conservation therapies. Houssami et al. [8] reported sensitivities of 65% vs 76% in those with a personal history of breast cancer vs. those without, with only 25–45% of recurrences detected on mammogram overall. Distortion from surgery and increased density from radiation therapies can affect the detectability of an early breast cancer.

Women of higher risk develop cancers at an earlier age, perhaps in denser breasts with tumours with atypical morphological mammographic features and a faster tumour growth rate. Mammographic screening is often performed annually from a younger age and is enhanced with magnetic resonance imaging in higher-risk women due to the reduced mammographic sensitivity reported in these women. MRI screening studies report sensitivities of 77–100% vs. mammographic sensitivities of 23–50% (Kuhl et al. [9], Kriege et al. [10], Leach et al. [11], Sardanelli et al. [12] and Warner et al. [13]). Women who have a high breast cancer risk due to TP53 mutations or Li-Fraumeni or A-T homozygotes should not undergo routine mammographic surveillance due to increased radiation sensitivity.

18.1.4 Limitations

2D mammography has limitations. The advent of digital mammography and full-field digital mammography has improved the visualisation of breast disease in comparison to film screen, especially in denser breasts; however, there has not been the improvement in cancer detection as had initially been hoped. In the DMIST study, Pisano [14] studied cancer detection in digital vs. film-screen mammography in 49,528 women in a multicentre, multivendor trial and found digital mammography to be more accurate in women <50 years with dense breasts or who were pre-/perimenopausal. The superimposition of structures can lead to the under-detection of breast malignancy. Fifteen to thirty percent of cancers may not be detected by screening mammography and present as interval cancers, between screens.

Interval cancer rates can be used as a measure of the effectiveness of a screening programme. It is not only a key

quality indicator of a screening programme but allows for the surveillance of individual radiologist performance and education. Interval cancers tend to be larger in size at presentation compared to screen-detected tumours and are more likely to have nodal metastasis. They tend to be invasive tumours with less than 5% of interval cancers being due to ductal carcinoma in situ (DCIS) in a study by Bennett et al. [15]. In a review, Housammi et al. [16] report that interval cancers have a prognosis similar to that of other symptomatic cancers. The review found approximately 25–45% of interval cancers were due to a false-negative read, i.e. a perception or misinterpretation error. These errors are usually minimal signs, where perception errors may be improved with double reading or CAD. In those cases clinically assessed, the errors may possibly be reduced by assessment guidelines and improved clinical decision making. True intervals, with no findings on the screening mammogram, account for 18–63% of cases. These cancers are hard to minimise except for the consideration of enhanced screening techniques or shorter screening intervals where feasible. Mammographically occult tumours at diagnosis account for 8–12%.

Studies have reviewed the characteristics of undetected and missed cancers (Table 18.1). Birdwell et al. [22] reviewed the data used in the Warren Burhenne et al. [20] study above to demonstrate 30% of the missed cancers were microcalcifications and 70% were masses (28% were spiculate or irregular). They reported breast density to be the second most common cause for missing a breast cancer (34% of cases) following a distracting lesion as the most common cause (44%). Bird et al. [18] also demonstrated missed cancers were less likely to have microcalcifications and more likely to be an increasing opacity in denser breasts.

Elmore et al. [23] report the overall sensitivity for screening mammography to be 75% with a specificity of 92.3%. The accuracy of mammography is variable, being limited by breast density and symptoms. Sensitivity and specificity in fatty breasts are as high as 87% and 97%, respectively.

The sensitivity of mammography is reduced in situations where the breast tissue may be obscured or distorted including cosmetic techniques as well as following breast-conserving treatments (Fig. 18.7). Heterogeneously and extremely dense breasts are an independent risk factor for breast cancer. Boyd et al. [24] discussed the increased risk of breast cancer in women with dense breasts to be four- to sixfold. The reasons for this are likely multifactorial, not only due to the reduced sensitivity of mammography which is only a "masking effect" but the increased volume of tissue

Table 18.1 Table of interval cancer studies with undetected and missed cancer analysis

Author	Year	Interval cancers reviewed	% with (actionable) signs on previous mammogram
Ikeda et al. [17]	1992	96	32
Bird et al. [18]	1992	320	24
van Dijck et al. [19]	1993	84	38
Warren Burhenne et al. [20]	2000	427	27
Brem et al. [21]	2003	377	32

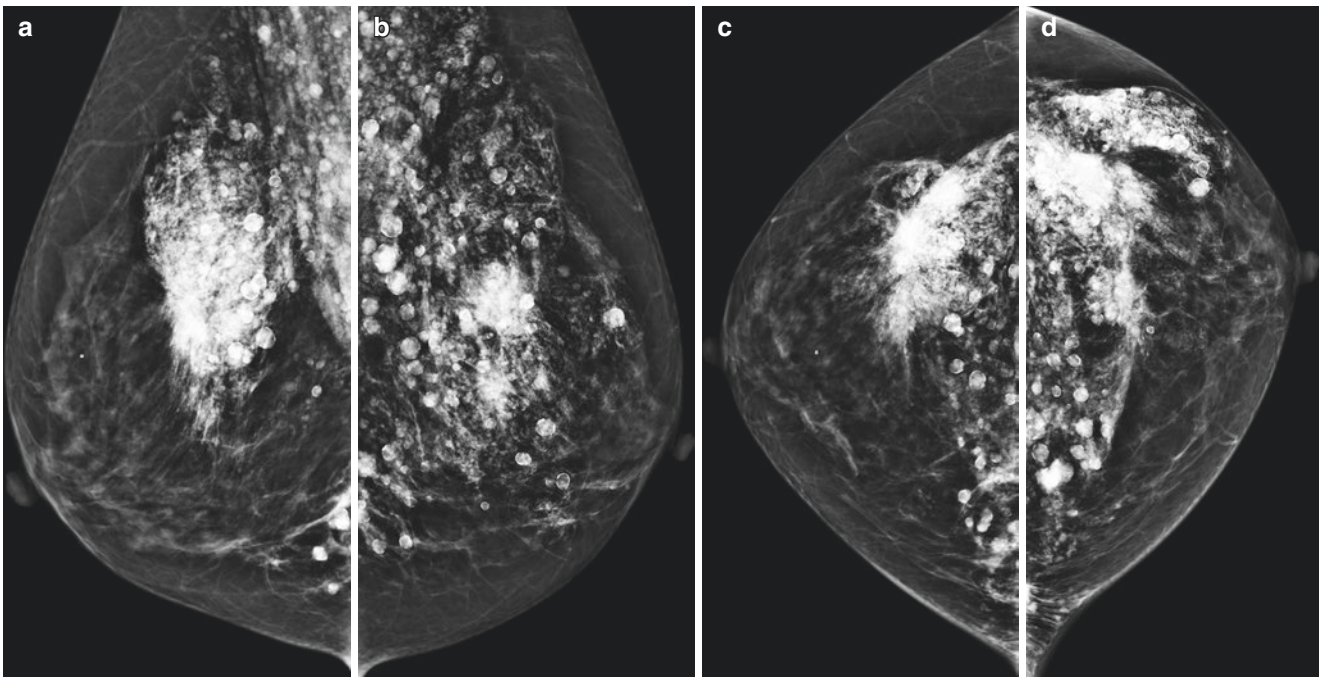


Fig. 18.7 (**a–d**) Silicone prevents the optimal visualisation of breast tissue reducing the sensitivity of the mammogram in the detection of early breast cancers

that may potentially undergo malignant change and possible underlying genetic predisposition. Dense breasts are associated with epithelial proliferation and stromal fibrosis. The review also looked at the effect of age, family history, diet, alcohol consumption, exercise and race on breast density and suggested age to have the strongest effect. Berg et al. [25] reported a sensitivity of 45% for the detection of malignant lesions in patients with extremely dense breasts. The study also examined cancer detection by tumour type. The sensitivity for invasive ductal cancer was 81% but 34% for invasive lobular cancer.

Mammographic sensitivity can be affected by a number of factors. The mammographic breast density and lifestyle factors affecting this, patient age, and reader experience affect the sensitivity (Banks et al. [26], Britton et al. [27]). Patient age has been shown to be a significant factor in mammographic sensitivity by multiple study groups. Kolb et al. [28] showed the sensitivity in younger women with dense breasts (<49 years) to be significantly lower than in older women with dense breasts. The mammographic accuracy may be affected by the context in which it is performed, as the reader may look for an abnormality on mammography to explain a presenting clinical symptom. Kavanagh et al. [29] reviewed 106,826 women presenting for routine screening and compared asymptomatic women to those with symptoms. The sensitivity in those with breast-specific symptoms was 80.8% vs. 75.6% in those asymptomatic but with significantly lower specificities of 73.7% and 94.9%, respectively.

The sensitivity of mammography is improved by double reading which may be carried out by two readers independently or together. This is common practice in the UK and other European countries. A systematic review by Taylor and Potts [30] has shown a significant increase of approximately 10% in cancer detection rate with double reading. Published data shows a variable effect on recall rate. In most studies specificity is not compromised. In cases where there is discordance between reader 1 and reader 2, the process of arbitration or consensus is used. Arbitration is performed by a single reader and consensus is by a panel. Duijm et al. [31] have shown the process of consensus and arbitration to be effective in recalling the majority of cases with cancer while minimising the recall rate.

Increasing the recall rate reduces the positive predictive value and the cost-effectiveness of mass screening. Schell et al. [32] performed a study of 1,872,687 mammograms in the USA and concluded recall rates between 6.7 and 10% (incident and prevalent screens) were optimal. Europe recall rates are between 3 and 10%. Smith-Bindman et al. [33] reported the average recall rate in the USA to be double that of the UK with no difference in cancer detection rates. This was explained in the study by multiple variations in screening practice between the UK and the USA. The UK national programme has robust radiology quality assurance in place including a minimum reading volume of 5000 mammograms per year per radiologist. The balance of sensitivity vs. specificity depends on multiple factors. It may be affected by litigation concerns; if the aim is to detect all cancers, i.e. maximise sensitivity, this will increase recall rates and reduce specificity.

The use of computer-aided detection (CAD) may optimise cancer detection by one reader where double reading is not available. The Computer-Aided Detection Evaluation Trial II (CADET II) compared single reader with CAD with double reading to show cancer detection/sensitivity between the two arms was similar, 87.2% and 87.7%, respectively (James et al. [34]). The specificity of double reading was higher at 97.4% in comparison to 96.9% with CAD which also resulted in a higher recall rate. There was no statistical difference between cancer subtypes although the CAD arm detected more in situ disease and smaller, higher-grade invasive disease. Pooled estimates from two meta-analyses of 27 studies with CAD by Taylor and Potts [30] concluded with the same results for cancer detection rates between CAD and double reading but a lower recall rate with double reading. It is reported that radiologist productivity was unaffected by CAD, perhaps due to radiologist experience in the final decision as to whether or not to recall (Brem et al. [21]). In addition to this, Freer and Ullissey [35] reported CAD not to affect the positive predictive value for biopsy.

CAD has been shown to be particularly effective in the detection of microcalcification clusters. The detection of microcalcification does not appear to be affected by breast density (Brem et al. [36], Birdwell et al. [22]). Distortions are the third most common mammographic sign for breast cancer and can be very subtle with appearances often mimicking overlapping tissues. Baker et al. [37] reviewed the performance of CAD on benign and malignant architectural distortions. In this study, CAD was not sensitive to subtle signs, detecting <50% of the cases. Among the cancers not detected by CAD, studies have shown a posterior location is more common; however, quadrants are equal.

CAD is a sensitive system; however, mammographic reading also requires the expertise of an experienced radiologist who is able to distinguish correctly between true-positive and false-positive prompts, ensuring that the correct areas are recalled without compromising specificity (Azavedo et al. [38]).

The positive predictive value (PPV) of mammography for malignancy and that for biopsy varies widely according to the mammographic sign, between 15 and 75%. The mammographic signs with the highest predictive value for malignancy are masses with a spiculated margin or irregular shape and linear microcalcifications in a segmental or linear distribution (Liberman et al. [39]). Lazarus et al. [40] reviewed the PPV by BI-RADS category with BI-RADS 4 and above recommended for biopsy and reported a PPV between 6% for BI-RADS 4a and 91% for BI-RADS 5 lesions.

The recall rate following screening reflects the specificity of mammography. The UK National Health Service Breast Screening Programme (NHSBSP) screened 2.08 million women in 2013–2014, detecting 8.6 cancers per 1000 screened of which 39.9% were 15 mm or less. The prevalent and incident screen recall rates were 7.9% and 3%, respectively. The false-positive rate in mammographic screening is a limitation which has significant morbidities related to anxiety and biopsy.

Screening is performed in otherwise (breast) healthy women; the harm vs. benefit must therefore be addressed. The Marmot review [41] of the benefits and harms in population mammographic screening reported for every life saved with breast screening, 180 women are screened or 235 women are invited to screening for 20 years. Screen-detected disease that may not otherwise have resulted in harm to the patient in her lifetime is known as overdiagnosis. The review estimated that 19% of screen-detected cancers are due to overdiagnosis or for each breast cancer death prevented, three women are overdiagnosed.

Overdiagnosis may include the detection of small low-grade invasive tumours and DCIS. The detection of DCIS has significantly increased with the advent of screening accounting for approximately 20% of screen-detected malignancy. The benefit of detecting DCIS against harm has been questioned. This is partly related to the very different natural history of low-grade DCIS in comparison to high-grade DCIS within the same disease category and the uncertainty about the progression of disease. A review of over 5.2 million screened women by Duffy et al. [42] provided evidence that the diagnosis and treatment of DCIS in screening are worthwhile and suggested that one less invasive interval cancer occurred for every three cases of DCIS detected.

The recognition of the variable sensitivity and specificity of mammography dependent on patient factors has led to the development of new techniques which enhance lesion detection compared with conventional mammograms. We discuss digital breast tomosynthesis and contrast-enhanced digital mammography.

18.1.5 Digital Breast Tomosynthesis (DBT)

DBT, sometimes called 3D mammography, provides the reader with images of the breast in thin slices and overcomes many of the interpretation problems of 2D DM due to overlapping normal tissue, sometimes referred to as "anatomical noise". The mammographic signs of breast cancer may be obscured, particularly in women with dense fibro-glandular breast tissue (Al Mousa et al. [43]), resulting in delay in the diagnosis of cancer. The UK national interval cancer data shows that up to 4000 women per annum (2.88 per 1000 screened) are diagnosed with breast cancer in the interval between screens (Offman and Duffy [44]). Conversely, superimposition of normal tissues may produce features on mammography which are suspicious for cancer and lead to unnecessary recall for further diagnostic tests. UK national screening data for 2012/2013 show that of 2.3 million women screened, 79000 (3.4%) without breast cancer were recalled to specialist diagnostic assessment clinics for further tests (Centre for Cancer Prevention [45]). DBT has been incorporated into the routine for further mammographic investigation of breast lesions in many centres replacing conventional spot compression views. Some centres in North America and Europe are already using DBT in addition to conventional 2D mammography to screen asymptomatic patients.

18.1.5.1 Technique

Tomosynthesis involves the movement of the X-ray tube in an arc during which data from multiple low-dose projection images are acquired. Between 9 and 25, low-dose projections are taken over an angular range of 15–50 degrees depending on the manufacturer. Images for viewing are reconstructed using either a filtered back projection or iterative method from the low-dose projection image data and are typically displayed as 1 mm thickness in-focus planes. The images may be viewed singly or as a series, similar to viewing a CT scan. The total dose is comparable to that of conventional full-field 2D digital mammography but depends on the manufacturer. Certain systems can also produce a reconstructed or synthetic 2D image from the tomosynthesis images which can reduce the total dose further by avoiding the need for conventional FFDM (Fig. 18.8).

18.1.5.2 Indications

This technique has been demonstrated to show increased accuracy in comparison to film-screen and full-field digital mammography (FFDM). This is particularly the case in the characterisation of soft tissue abnormalities. There does not, however, appear to be an advantage of DBT in the assessment of microcalcification (Spangler et al. [46]). The TOMMY trial (TOMosynthesis with digital MammographY) reported DBT specificity was highest for distortions and lower for microcalcifications (Gilbert et al. [47]). Michell et al. [48] demonstrated an accuracy of 97% when interpreting using DBT in comparison with 90% with 2D FFDM. Cancer visibility has been shown to be superior with DBT. Studies have shown single-view MLO projection DBT to be more accurate in the detection of tumours than two-view DM (Andersson et al. [49], Svahn et al. [50]).

The improved visibility of the margins of circumscribed soft tissue lesions may enable readers to predict the likelihood of malignancy as presented by Wasan et al. [51]. The addition of DBT improves lesion conspicuity, margin analysis and the detection of additional abnormalities.

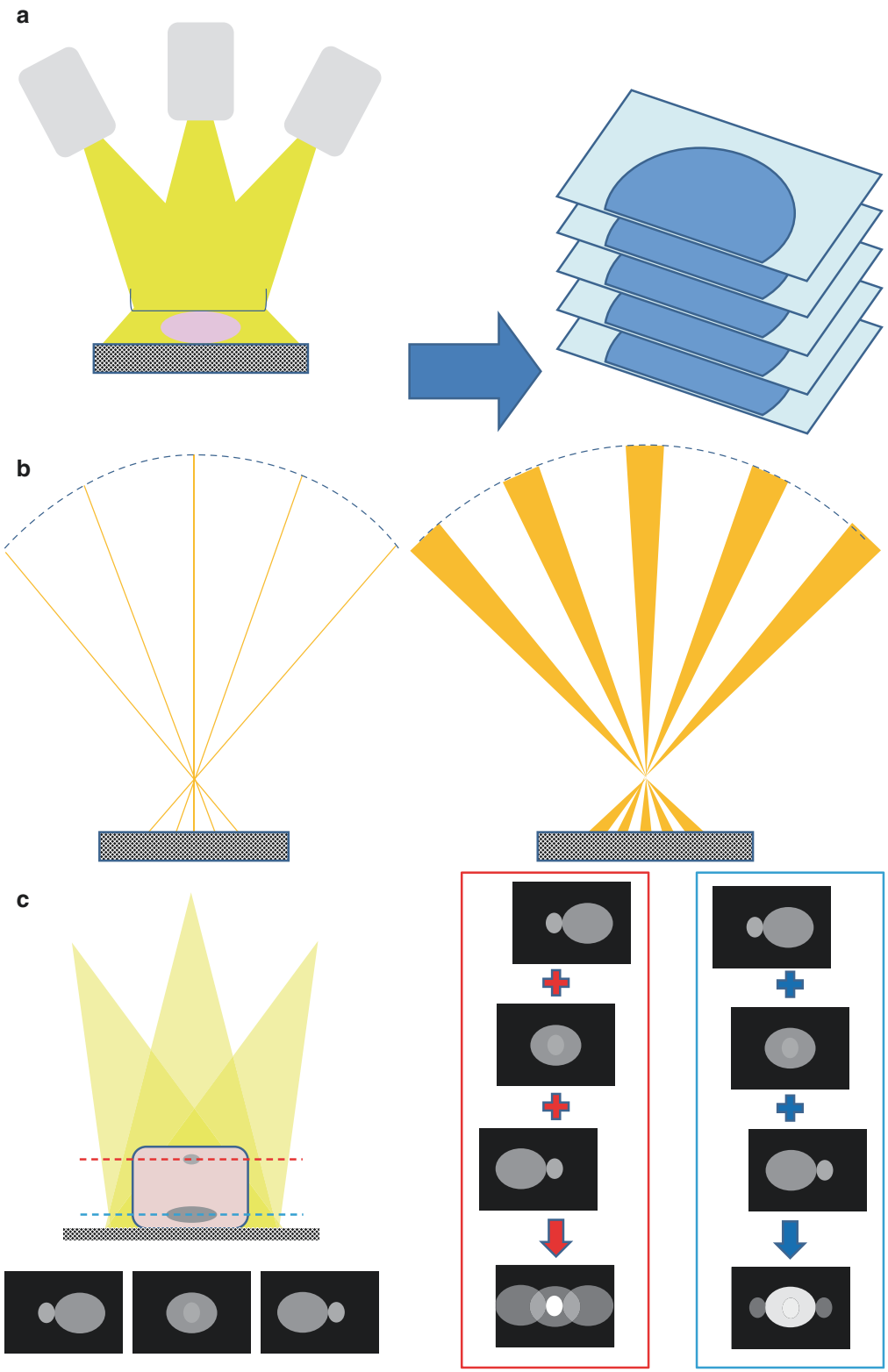


Fig. 18.8 (**a**–**c**) Illustrations courtesy of Dr. Celia Strudley, The Royal Marsden Hospital, London. (**a**) Illustrates how DBT acquires multiple images through an arc. The focal planes taken from sequential depths are then stacked approximately 1 mm apart. (**b**) These images demonstrate tube motion. The step and shoot tube movement has a small focal spot and produces sharp images but is slower to acquire images. The continuous tube movement elongates the focal spot causing blurring in the direction of the motion but is faster and has smoother motion. (**c**) This illustrates the simple back projection reconstruction method used by some DBT systems

The role of DBT in the diagnostic workup of soft tissue masses, distortions and asymmetrical densities is established. The reported advantage is the improved ability to predict malignant lesions without increasing the false-positive rate (Morel et al. [52] and Zuley et al. [53]). Studies have shown fewer benign biopsies and short-term follow-up are recommended with the use of DBT. This results in a significant improvement in the accuracy of diagnostic assessment. There

are further advantages to using this technique over coned compression views as visualisation of the whole breast improves detection of multifocal disease.

Gur et al. [54] demonstrated an improved performance with DBT including demonstrating lesion location and multifocal lesions; however, this was at the cost of an increased false-positive rate which was justified by the increased true positives (Figs. 18.9, 18.10, 18.11, 18.12, 18.13).

18.1.5.3 Potential Use in Screening

A topic which is of interest in research at the time of writing this chapter is the possible use of DBT in the routine

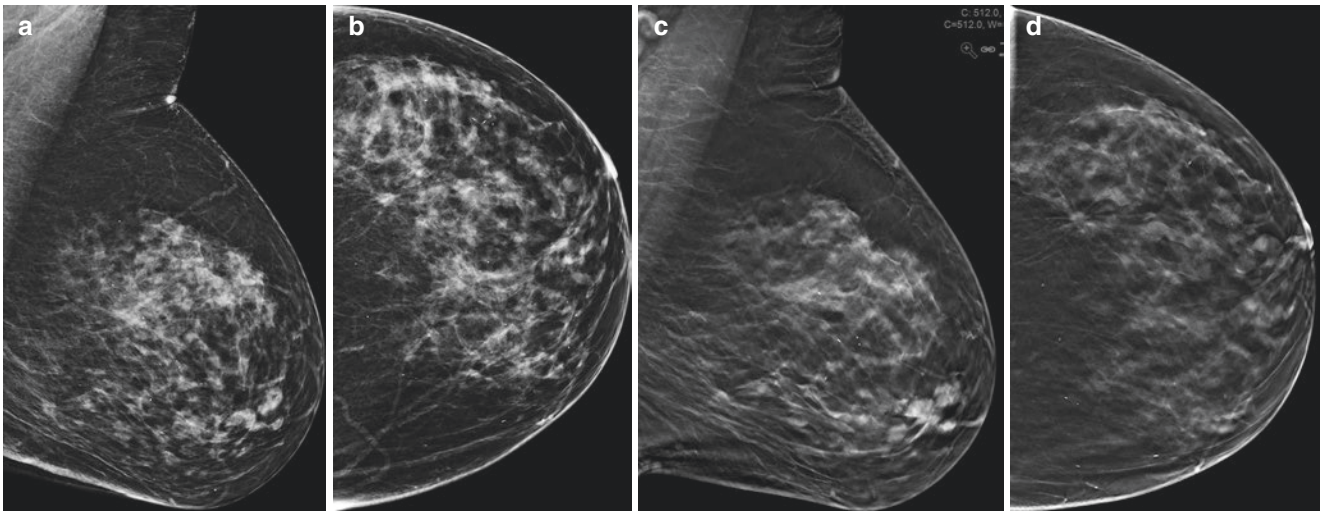


Fig. 18.9 (**a**–**d**) DBT increases the conspicuity of a lesion enabling a sign change in this case from a circumscribed mass to a spiculate mass. This case is a screen-detected lymph node negative 12 mm grade 2 invasive lobular carcinoma recalled to assessment on the (**a**) LMLO and (**b**) LCC 2D FFDM, which demonstrated a circumscribed mass in the lower outer breast. (**c**) LMLO (**d**) LCC DBT demonstrates a spiculate lesion improving reader confidence and BI-RADS 5 score from the initial BI-RADs 4a based on the 2D images

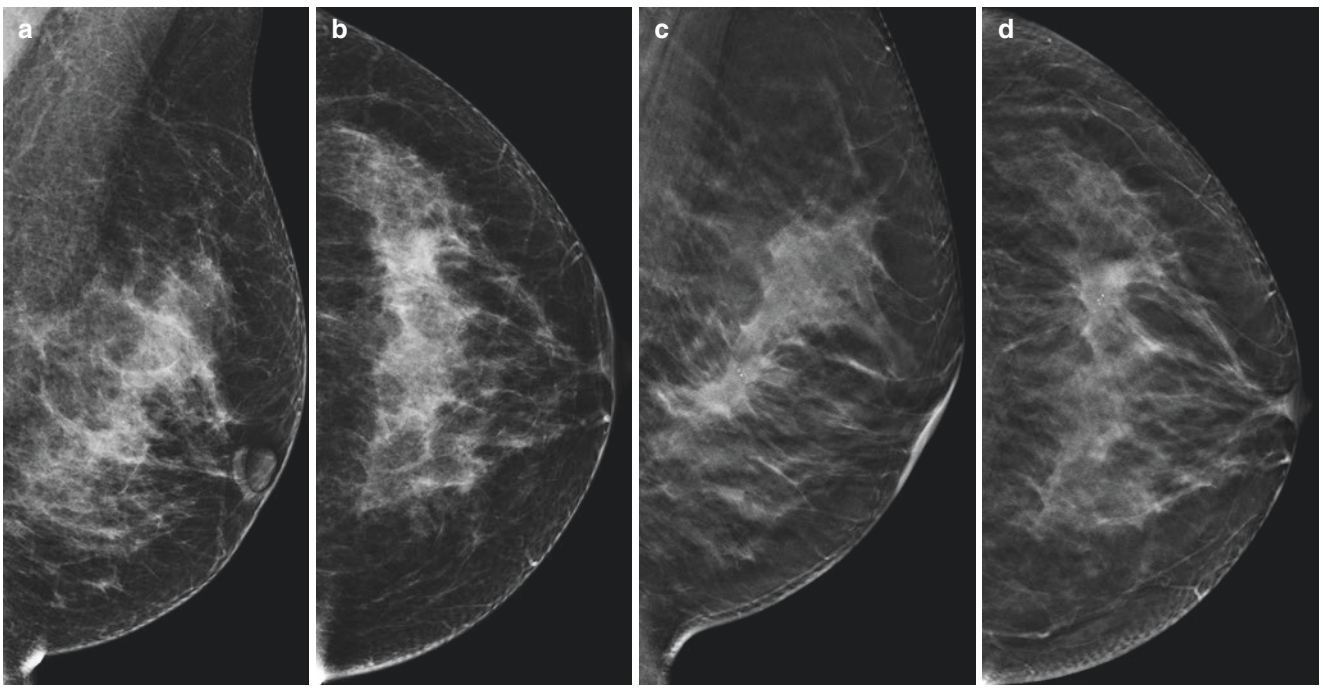
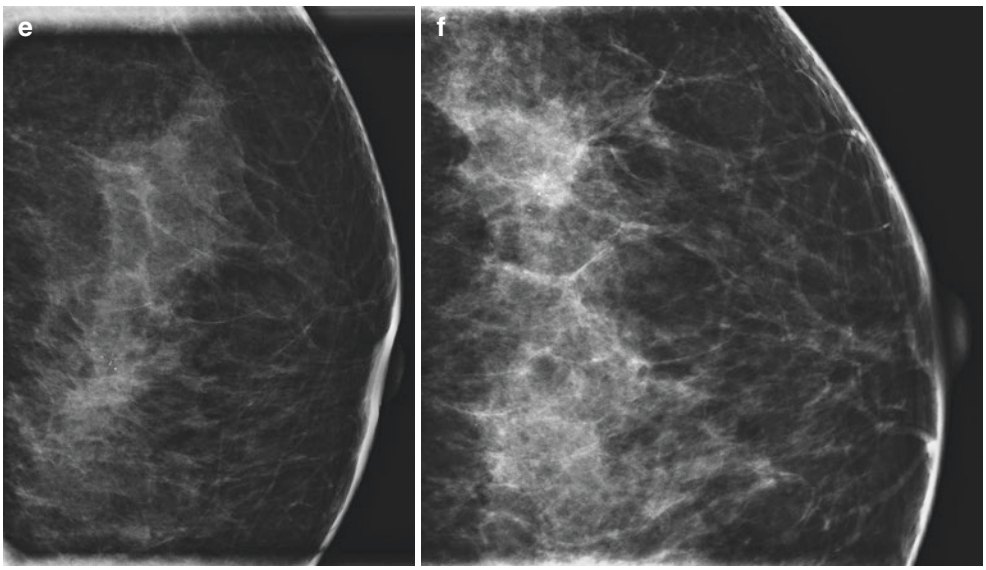


Fig. 18.10 (**a**) LMLO 2D FFDM, (**b**) LCC 2D FFDM, (**c**) LMLO DBT, (**d**) LCC DBT, (**e**) left lat magnification view and (**f**) left CC magnification view. The 2D FFDM screening mammograms (**a**, **b**) demonstrate microcalcification which underwent supplementary views. DBT (**c**, **d**) demonstrates a spiculate mass which is occult on the 2D. The magnification views (**e**, **f**) demonstrate the microcalcification and subtle increased density. Final pathology demonstrated a grade 2 invasive ductal carcinoma with axillary lymph node involvement

Fig. 18.10 (continued)



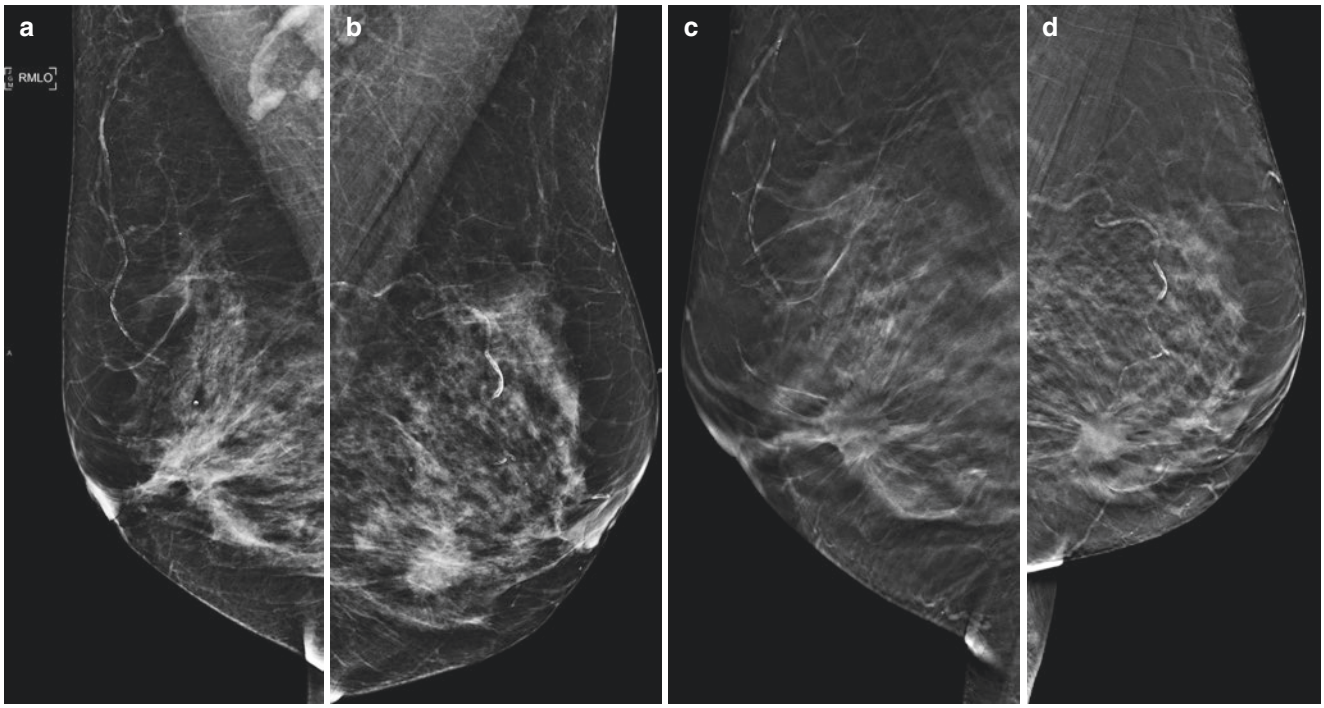


Fig. 18.11 (**a**) RMLO 2D FFDM, (**b**) LMLO 2D FFDM, (**c**) LMLO DBT and (**d**) RMLO DBT. The 2D FFDM demonstrates bilateral breast cancers. The *left breast cancer* was a grade 2 invasive ductal carcinoma with spread into the axillary lymph nodes. The *right breast cancer* was a grade 2 invasive lobular carcinoma. However, the extent of the breast tumour is more clearly appreciated with the DBT especially the *right breast tumour*

screening of women for breast cancer. The radiation dose of DBT is similar to that of established 2D DM which invites the possibility of this technique being used for population screening. Studies of DBT used in addition to 2D DM have shown a significant increase in invasive cancer detection rates with no significant effect on DCIS. Further prospective trials are underway in North America (TMIST), Norway (Oslo trial), Italy (Storm trial), Sweden (Malmo trial) and the UK (PROSPECTS).

The Malmö Breast Tomosynthesis Screening Trial (MBTST) showed an increase in sensitivity in cancer detection with single-view DBT vs. two-view mammography. This

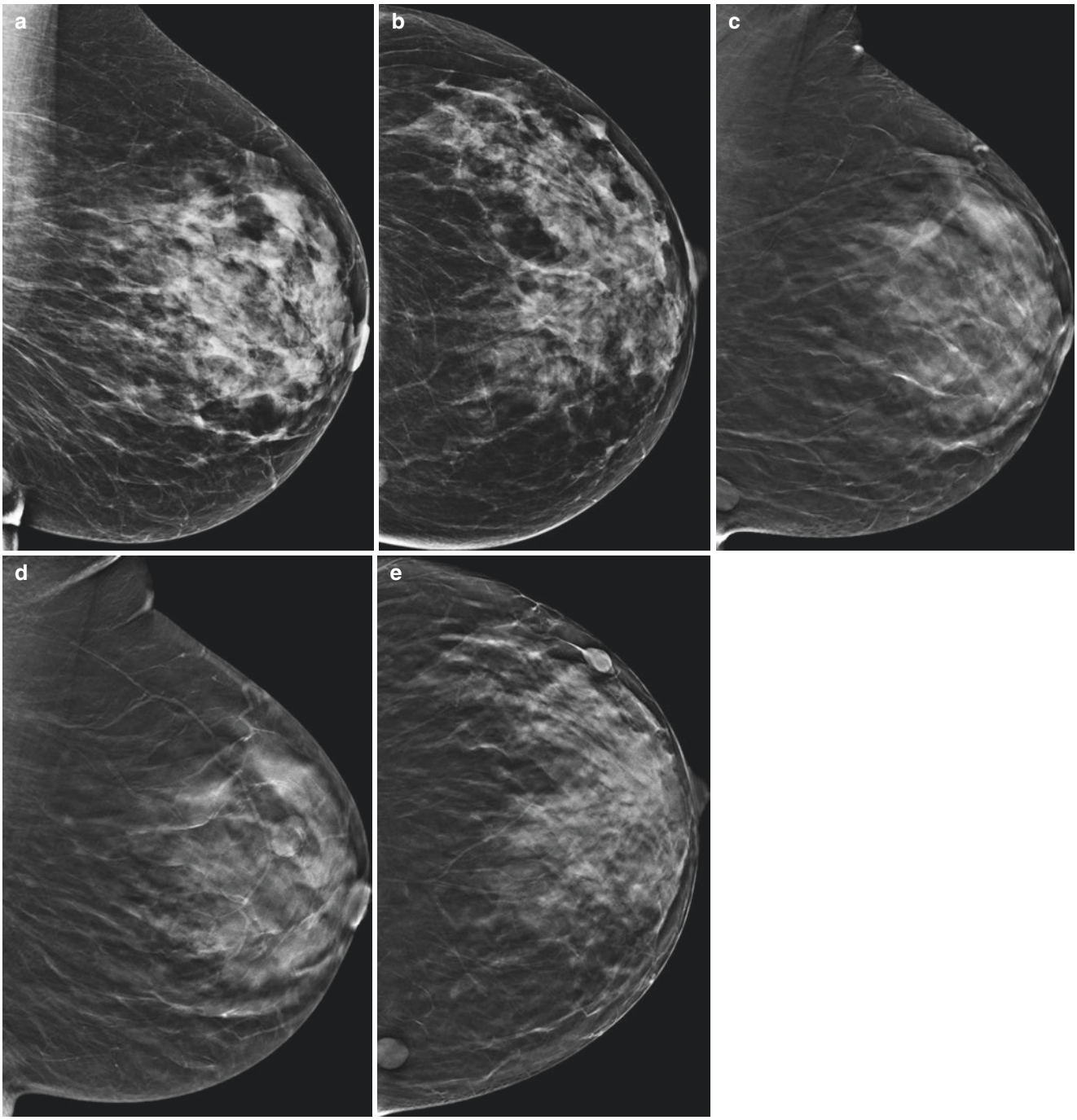


Fig. 18.12 (**a**) LMLO 2D FFDM, (**b**) LCC 2D FFDM, (**c**) LMLO DBT, (**d**) LMLO DBT and (**e**) LCC DBT. (**a**–**b**) 2D FFDM images of the left breast demonstrate BI-RADS 3 breast density with a nodular parenchymal pattern and multiple densities with partial visualisation of the margins. (**c**–**e**) DBT images enable clear visualisation of 100% of the margins of these multiple lesions which were biopsy-proven fibroadenomas. The DBT images enable confident diagnosis of benign lesions which would require no further investigation

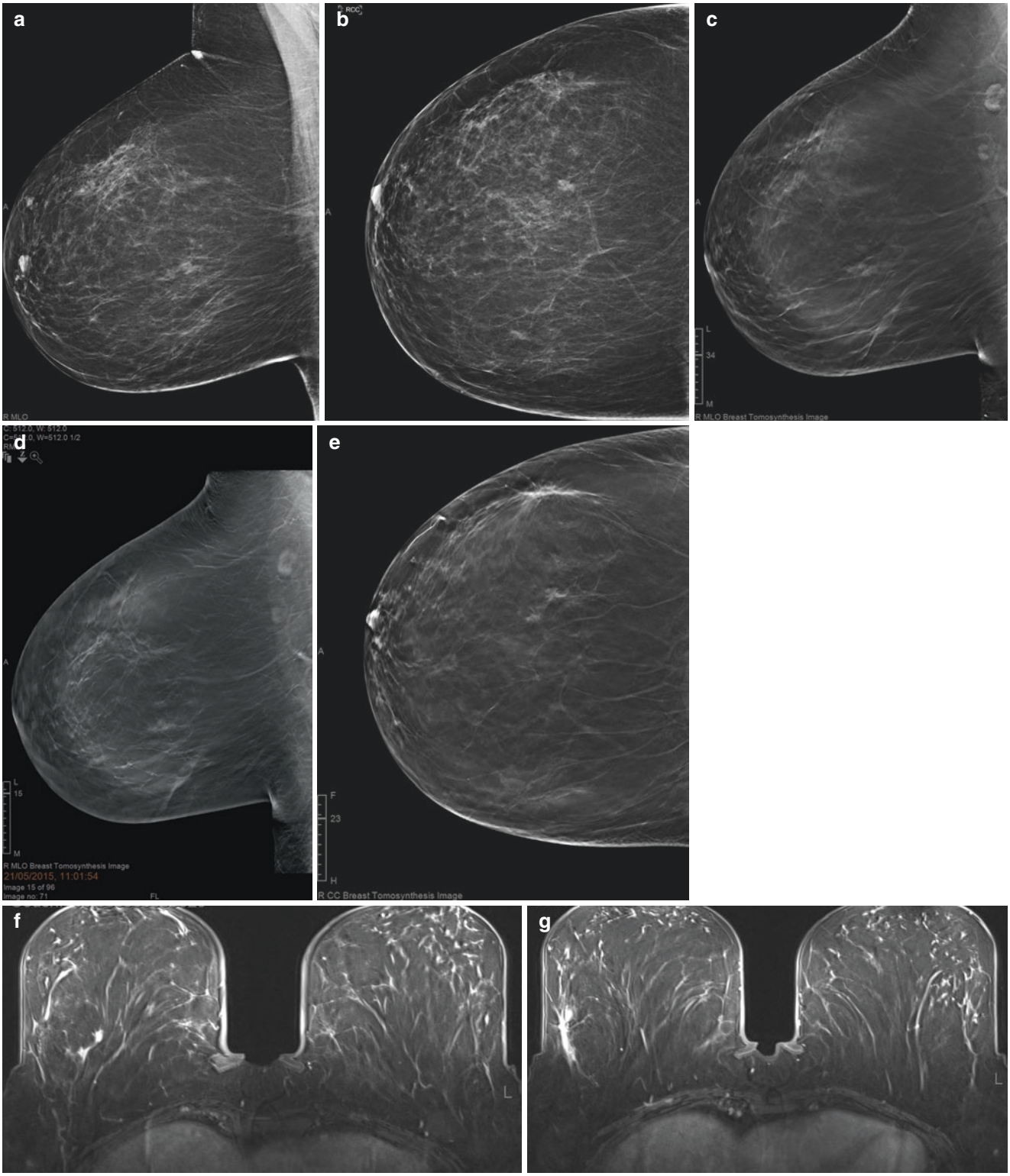


Fig. 18.13 (**a**) RMLO 2D FFDM, (**b**) RMLO 2D FFDM, (**c**) RMLO DBT, (**d**) RMLO DBT, (**e**) RCC DBT, (**f**) axial 1 min post-contrast MRI and (**g**) axial 1 min post-contrast MRI. This case illustrates the detection of multifocal disease in a screen-detected multifocal grade 2 invasive lobular carcinoma. The asymptomatic woman was recalled for a new central irregular-shaped mass on routine 2D screening mammogram (**a**–**b**). DBT at the time of assessment demonstrated the central mass as two spiculate lesions on (**c**) RMLO DBT and (**e**) RCC DBT. Further to the recalled lesions is a distortion in the *right upper outer breast* not appreciated on 2D FFDM but detected on the (**d**) RMLO DBT and (**e**) RCC DBT. Post-contrast MRI images (**f**–**g**) illustrate the three tumours

study design takes into account the practical issues of increased radiation dose and radiologist reading time, increased costs and increased data storage by the use of a single MLO DBT view as opposed to two-view DBT. This may enable DBT to be more feasible in the context of mass screening (Table 18.2).

A further area of interest is the reconstruction of tomosynthesis images into a synthetic 2D mammogram which may be comparable to conventional 2D FFDM (Fig. 18.14). The clear advantage is the dose reduction as a result of not needing to perform conventional 2D digital mammography. Skaane et al. [58] demonstrated cancer detection using conventional 2D FFDM vs. reconstructed synthetic 2D imaging showing no statistical difference when using the most current software. They also reported that a single read of synthetic 2D with DBT detected more cancer than double read FFDM.

DBT may demonstrate subtle lesions which are not visible using conventional 2D mammography or ultrasound. DBT-guided biopsy/intervention is now available. This technique can be used not only for the biopsy of subtle lesions seen on DBT alone but also for the same indications as stereo-guided biopsy or wire insertion.

There are challenges and some uncertainties related to the implementation of DBT in routine screening. There is a continued requirement for 2D or synthetic 2D mammogram images. There is uncertainty about the continued effect of DBT on sensitivity and specificity following the first "prevalent" DBT screen.

There are mixed results from studies for recall rates as well as a possible increase in single reader/discordant cancer detection. This would imply there is a learning curve with the perception and interpretation of tomosynthesis images. It is also possible that DBT may further increase overdiagnosis rates. The Oslo Tomosynthesis Screening Trial showed increased cancer detection with biologically significant disease and no increase in the detection of DCIS. The improved performance of DBT was seen in all breast densities including fatty breasts.

There may be increased costs associated with the technology, image data storage and longer reading time (Bernardi et al. [59]). The increase in radiologist reading time which may improve with experience; however, this may limit reading volumes possible by a reader as well as have cost implications. The Oslo screening study estimated a reading time of 45 s for 2D and 91 s with DBT. It is possible that reading times are longer than this depending on experience, equipment and hanging protocols. The quality assurance workload for radiographic and physicist staff is increased as is the time to perform studies. This again has implications for a high volume screening workloads and cost. The Oslo study estimated an increase of 10 s per view for an experienced mammographer to perform DBT in combination with the 2D DM. A centre using tomosynthesis will also consider the increase in data storage capacity required (an approximation of 20 MB for 2D vs. 2000 MB for DBT).

CAD may play a role in DBT screen film reading. This is being investigated in an arm of the Oslo trial; however, Kilburn-Toppin and Barter [60] have suggested CAD would remain a supplementary tool only and will not substitute radiologist reading.

18.1.6 Contrast-Enhanced Spectral Mammography

This new technique has been described as a more accessible breast MRI study. Contrast-enhanced spectral mammography (CESM) utilises tumour angiogenesis in a similar way to contrast-enhanced breast MRI (CE-MRI). CE-MRI is currently accepted as the most sensitive imaging technique for detecting and staging breast cancer.

18.1.7 Technique

There are two recognised techniques used in CESM: temporal subtraction and dual energy. Both techniques involve the administration of iodinated contrast at a rate of approximately 3 mL/s.

Table 18.2 Table of prospective DBT Breast cancer screening studies with double reading

Study	No. of patients	Design	Recall	False-positive rate	Cancer detection rate	No. of cancers/1000 2D vs. 3D(+2D)
Oslo (Interim results: Skaane et al. [55])	12,621	2 view (V) 2D vs. 2 V 2D + 2 V 3D	Increase 32%	Decrease 13%	Increase 31%	6.1 vs. 8.0
Malmö Lang et al. [56]	7500	2 V 2D vs. 1 V 3D	Increase 43%		Increase 43%	6.3 vs. 8.9
STORM (Interim results: Ciatto et al. [57])	7292	2 V 2D vs. 2 V 2D + 2 V 3D		Decrease 17.2%	Increase 51%	5.3 vs. 8.1

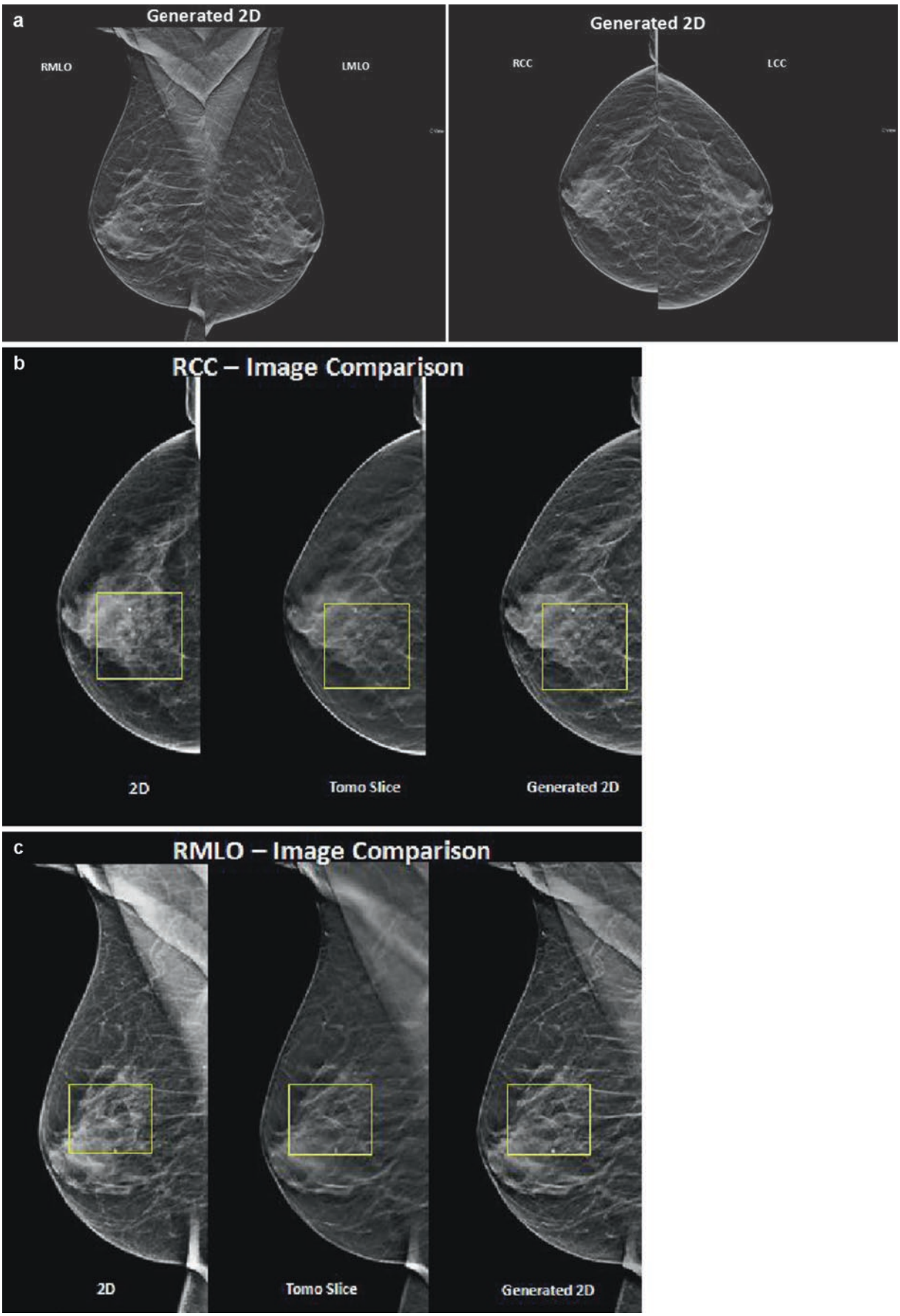


Fig. 18.14 (**a**–**c**) Images courtesy of Hologic, Inc. This is a case of invasive ductal carcinoma seen as an architectural distortion with associated microcalcification seen on the 2D, DBT and reconstructed 2D

Temporal subtraction acquires images with the patient's breast held in a single light compression which limits movement and minimises compression of the blood vessels. A pre-contrast image is taken and contrast is administered via power injector. Multiple high-energy images are taken over several minutes. The pre-contrast image is subtracted from the post-contrast images. Software enables the kinetic analysis of a lesion as with CE-MRI. A single contrast dose is required for each analysis with one breast in one projection.

In the dual-energy technique, images are acquired using high- and low-energy exposures following contrast administration via a power injector. A subtraction technique is then used to suppress the background of fibro-glandular tissue and fat, enabling clear demonstration of enhancing tissue. The light compression used is to avoid motion but enable blood flow. The shorter time to acquire the images in this technique limits motion artefact compared with the temporal subtraction technique. Images in MLO and CC projections of both breasts can be taken with a single contrast injection over 5–10 min. The dose is between 20 and 50% higher than that of a single mammographic view. Kinetic analysis is not possible with this technique (Fig. 18.15).

18.1.8 Possible Indications

This technique is predominately used in the research setting at the time of writing this chapter, although some centres have begun using this technique in diagnostic clinics and possibly in the screening of high-risk patients. It is proposed this technique can be used with similar clinical indications as CE-MRI, although this has yet to be established. These include:

- The staging of diagnosed breast cancer particularly in women with dense breasts or distracting benign lesions. This includes evaluating disease extent as well as multifocal disease and contralateral disease which may be mammographically occult.
- The investigation for equivocal breast lesions.
- An alternative to CE-MRI for the screening of high-risk family history screening.
- The detection of a primary tumour in patients with positive axillary lymph nodes and negative standard mammography and ultrasound.
- The potential evaluation of treatment response for breast tumours undergoing neoadjuvant chemotherapy or primary hormonal therapy.
- The investigation of recurrent disease where posttreatment changes may make mammographic interpretation challenging.

The sensitivity of CESM has been proven to be comparable to that of CE-MRI in the detection of index tumours. Jochelson et al. [61] demonstrated CESM and CE-MRI to both exhibit a sensitivity of 96% in detecting the index tumour (81% with conventional mammography) using the dual-energy technique. However, CE-MRI was significantly more sensitive in the detection of multifocal and multicentric additional ipsilateral disease than CESM (56% vs. 88%, respectively). CESM has been shown to give an accurate size measurement/disease extent compared with the final histology (Dromain et al. [62] and Jochelson et al. [61]).

The improved sensitivity of CESM in comparison to non-enhanced mammography is seen in all breast types (Diekmann et al. [63]). The higher spatial resolution of mammography to MRI enables a more critical analysis of lesion morphology as well as the visualisation of microcalcification, not visible with CE-MRI. This is evident by the high rates of specificity reported with CESM than with CE-MRI. Jochelson et al. [61] reported a PPV of 97% for malignancy with CESM compared with 85% for CE-MRI. The improved specificity with CESM may reduce the false-positive findings and benign biopsies secondary to CE-MRI as well as avoiding the practical limitations of scheduling the study by the woman's menstrual cycle, which does not affect CESM. Studies using the temporal subtraction technique have shown very variable kinetic analysis curves for malignant lesions that do not reflect those seen with CE-MRI. However, the enhancement patterns and distribution seen with CESM may mimick those seen with CE-MRI. Jong et al. [64] describe rim-like enhancement, irregular masses and inhomogeneous and linear enhancement of malignancies using a temporal subtraction technique.

CESM would be more cost-effective and time efficient in the diagnostic clinic setting where it may be performed along with standard mammography and ultrasound. CESM may also enable a more accessible means to biopsy and localise disease occult by conventional imaging methods.

The limitations of CESM include the contraindications for contrast administration which are documented contrast allergy, renal insufficiency and the relative contraindications of pregnancy and lactation. Similarly, potential complications include those of intravenous access and contrast reactions. Tumour conspicuity may be affected by the possible reduced blood flow and hence subsequent enhancement from compression of the breast, the reduced contrast resolution and the effect of enhancing overlying fibro-glandular tissue. The limitations of this technique in enhanced high-risk screening instead of CE-MRI would be secondary to the ionising radiation dose.

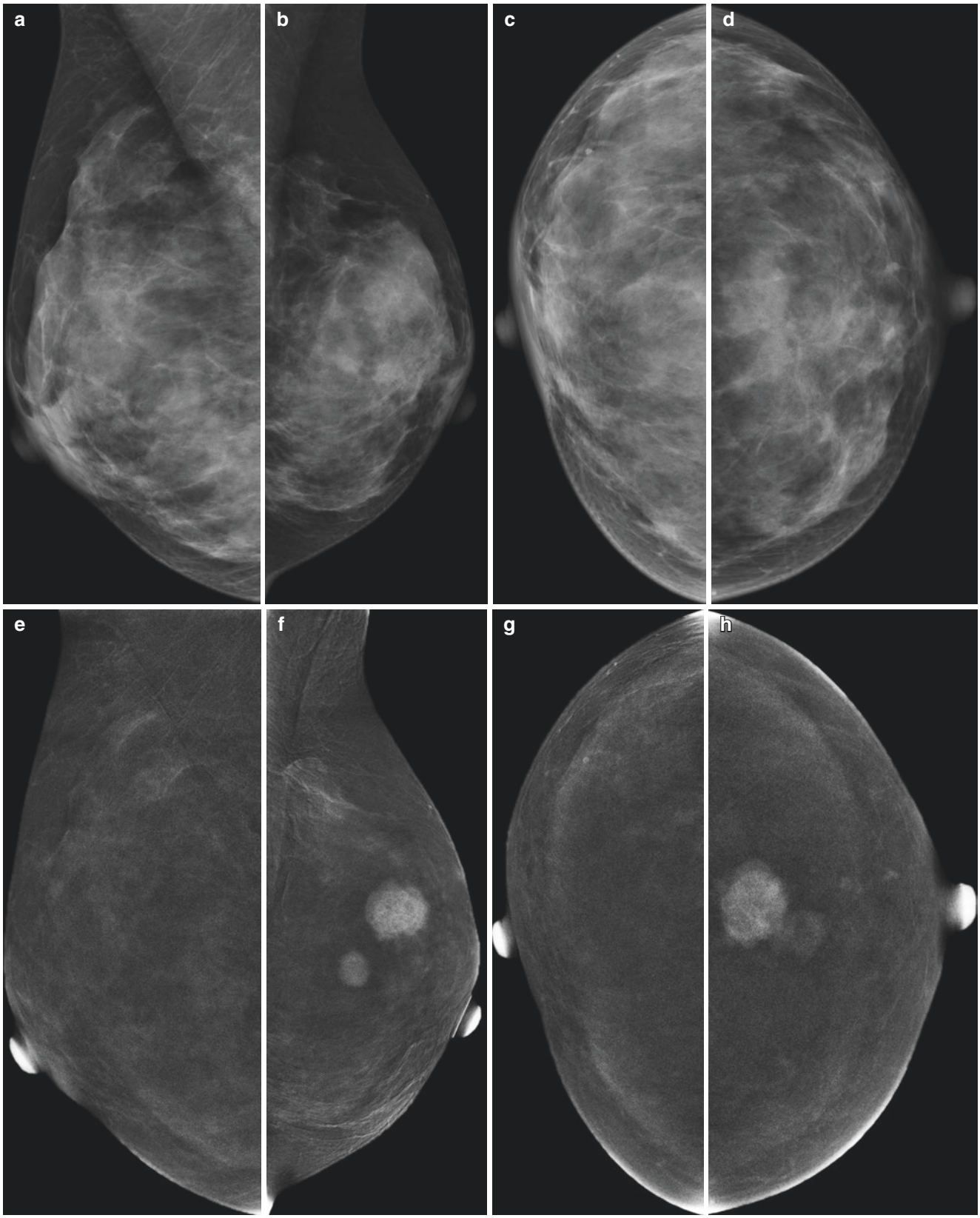


Fig. 18.15 (**a**–**h**) Images courtesy of Dr. Sarah Tennant, Nottingham University NHS Hospital and the Nottingham Breast Institute. (**a**–**d**) are the low-energy images of BI-RADS 4 dense breasts. The smaller tumour is seen on the (**b**) LMLO 2D FFDM with only a subtle increased density seen within the upper breast representing the larger tumour. This area of increased density may easily be overlooked on 2D DM. The post-contrast subtracted images (**e**–**h**) clearly demonstrate two grade 3 invasive ductal cancers measuring 19 and 13 mm on final histology without overlapping breast tissue or distracting lesions

18.2 Ultrasound

Viviana Londero, Chiara Zuiani, Martina Zanotel, and Massimo Bazzocchi

Abstract Breast ultrasound (US) is an indispensable tool in breast imaging, and, thanks to advances in US technology, its role is currently not limited to distinguish cystic from solid masses. In fact, a variety of new technical developments, the use of high-resolution probes and the application of a standardised BI-RADS US lexicon have improved characterisation of solid breast masses.

The authors provide an overview of recent advances in US technology, highlighting the applications of breast US in clinical practice. A description of BI-RADS US lexicon and the semiotic of cystic and solid breast lesions will also be presented.

Keywords Breast ultrasound • BI-RADS US • Breast disease • Colour Doppler • Elastography • 3D US • ABUS

18.2.1 Introduction

Breast ultrasound (US) has become an indispensable tool in breast imaging, usually complementary to mammography and magnetic resonance (MR) imaging. Although is under discussion the use of whole-breast US as supplement screening tool in women with dense breasts, its primary and routinely role is the characterisation of lesions detected at mammography, MR imaging or clinical breast examination [65].

The first clinical applications of breast US in the 1960s exploited the ability of US to distinguish cystic from solid masses, with the result that cystic benign lesions did not require further workup [66]. However, the poor image contrast and fair resolution of the first US machines did not allow further differentiation among solid breast masses [65]. Over the next decades, the advances in US technology, the development of high-frequency US transducers and the application of a standardised BI-RADS US lexicon allowed to obtain more detailed information about shape, orientation, margins, lesion boundary, echo pattern and posterior acoustic features of breast lesions, with the result to improve lesion conspicuity in the background of surrounding parenchyma and to improve characterisation of solid breast masses.

The US semiotic was subsequently ameliorated after the publication of Stavros' landmark study in 1995 [67], demonstrating that high-resolution greyscale US imaging could accurately distinguish benign from malignant lesions. In particular, Stavros et al. [67] developed a classification system for solid breast masses that achieved a 98.4% sensitivity and a 99.5% negative predictive value for malignancy. Among benign US features, the author included ellipsoid shape, gentle bi- or tri-lobulations, a thin echogenic capsule and a homogeneously echogenic echotexture [67]. Malignant US features included spiculated or angular margins, "taller-than-wider" orientation, marked hypoechogenicity, posterior acoustic shadowing and microcalcifications [67].

These important results were confirmed by other authors [68, 69], and the "Stavros' sonographic features" or "Stavros' criteria" are currently considered the cornerstones in the US assessment of breast solid lesions. Nowadays, these US signs ("descriptors") are widely illustrated and validated by the ACR BI-RADS US (Breast Imaging-Reporting and Data System) [70].

18.2.2 Conventional 2D Ultrasound, Compound Imaging and Harmonic Imaging

In addition to traditional greyscale US examination (B-mode), complementary tools now available in almost US units include compound imaging and harmonic imaging that can be used to ameliorate image contrast and resolution. Colour Doppler and power Doppler (more sensible to low-flow vessels) analysis allows to assess vascular architecture of the lesion and of surrounding breast tissue.

18.2.3 Compound Imaging and Harmonic Imaging

High-quality 2D US in combination with a precise examination technique, including radial and anti-radial scanner movements and a moderate tissue compression, is the basis for improving lesion conspicuity and for detecting small breast lesions and early-stage breast cancers.

Compound imaging and harmonic imaging represent 2D US technical advances introduced to ameliorate the image contrast and resolution, and these tools should be routinely used during US examination in order to optimise image quality. Compound imaging, with the use of an electronic beam steering, allows to acquire multiple US images from different angles, providing in real time a single final image that represents the average of these multiple images [71, 72]. The main advantage of compound imaging is that returning echoes from real structures are enhanced, with improved contrast resolution, resulting in a better definition of lesion margins, echogenic halos, posterior and lateral borders and better visualisation of microcalcifications and subtle architectural distortions [72]. Therefore, compound imaging is

able to reduce some advantageous artefacts such as artefacts behind Cooper's ligaments, but, at the same time, some helpful artefacts typically used as semiotic signs to differentiate cystic from solid nodules (such as posterior acoustic enhancement or shadowing) can be eliminated. Therefore, caution is necessary when applying this technique to lesion analysis.

Another modern algorithm, called "speckle reduction imaging" (SRI), that can be used simultaneously with compound imaging can help to enhance contrast and to optimise image quality during US examination.

To explain harmonic imaging, we have to consider that when US pulses travel along breast tissue, they can be distorted, creating harmonic frequencies [72]. The returning US signals may therefore contain both the original fundamental frequency and its multiples or harmonics. In harmonic imaging, the higher harmonic frequencies are filtered and used to create the greyscale US image with improved contrast, whereas lower-frequency artefactual internal echoes (typical of fluid components) are eliminated. As a consequence, the harmonic technology provides a better characterisation of simple cysts (especially if small) (Fig. 18.16) and a better definition of subtle lesions. Harmonic imaging also improves lateral resolution and assessment of lesion margins (Fig. 18.17).

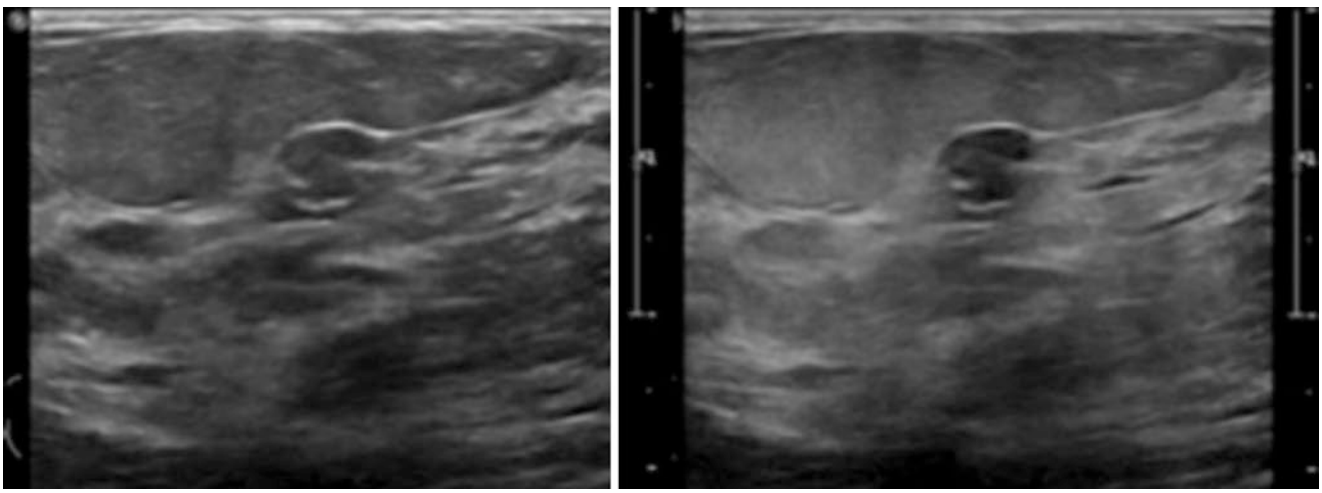


Fig. 18.16 Harmonic imaging. Solid hypoechoic lesion (fibroadenoma). Harmonic imaging (*right figure*) shows more accurately lesion margins and allows a significant increase of signal to noise ratio

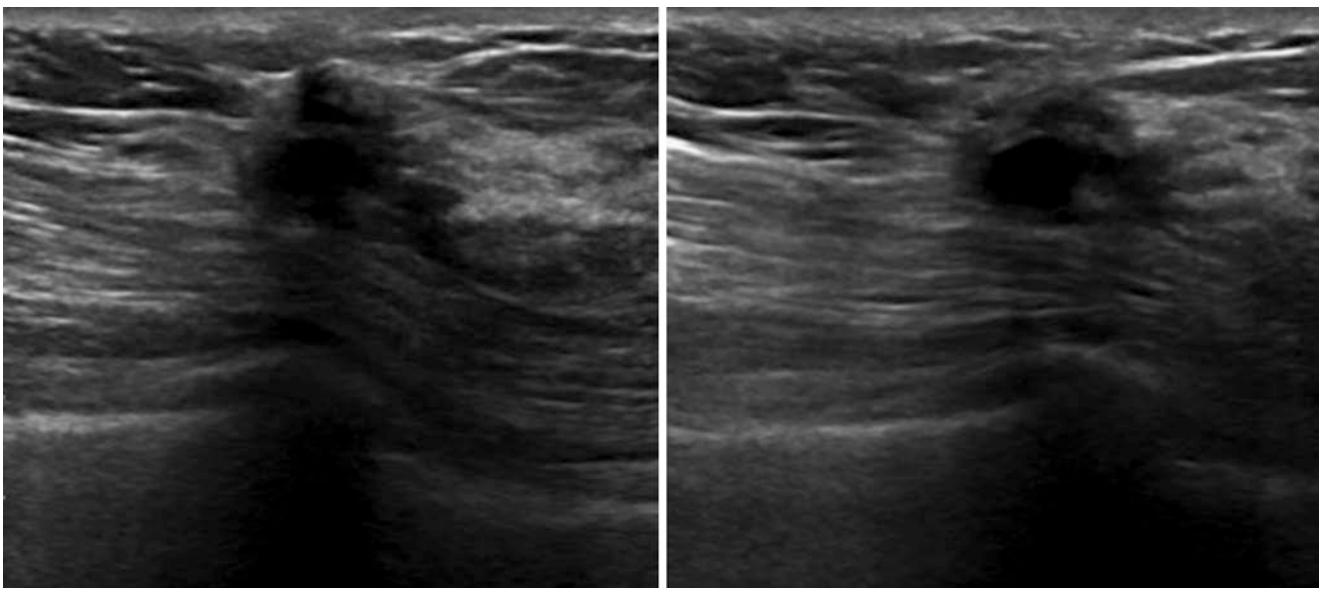


Fig. 18.17 Harmonic imaging. Hypo-anechoic lesion in woman with previous quadrantectomy; harmonic imaging (*right figure*) highlights with greater accuracy the oval morphology and partially circumscribed margins defining the cystic nature of the lesion (liponecrosis)

18.2.4 Colour Doppler and Power Doppler

With improvements in breast US technology, colour Doppler and power Doppler have become complementary tools to greyscale breast ultrasound, giving information about vascularity of solid lesion and of surrounding breast tissue. Power Doppler has been seen to be more sensible to low-flow vessels typical of breast lesions that, generally, require a moderate tissue compression in order to prevent occlusion of vessel lumen.

First applications of colour Doppler in the mid-1990s and early 2000s demonstrated that the presence of increased vascularisation within a solid breast mass could raise the suspicion of malignancy. In fact, Cosgrove et al. [73] in 1993 found that 99% of malignant lesions exhibited significant vascularisation at colour Doppler compared to only 4% of benign lesions. Sehgal et al. [74] found that benign lesions were two times more vascular than the surrounding tissue, compared to five times higher vascularity of malignant lesions.

Further studies in subsequent years did not confirm these initial promising results and concluded that colour Doppler should not be considered a reliable tool in the assessment of breast disease, because it could not accurately differentiate benign from malignant lesions [75, 76]. Although consensus has not been reached on usefulness of Doppler analysis, nowadays these vascular tools should be an integral part in every breast ultrasound practice.

In Gokalp's study [76], power Doppler criteria predictive of malignancy included hypervascularity, penetrating vessels within a solid mass and neovessels with branching-disordered course; however, the authors did not find any contribution to BI-RADS US, with the addition of power Doppler ultrasonography and spectral analysis.

Thanks to higher sensitivity to low-flow vessels, power Doppler may be useful in distinguishing a "centripetal" vascular pattern, generated by anomalous neovessels in malignant lesions, from the "centrifugal" vascularity (with a parallel artery and vein in the periphery or inside a lesion), predominantly associated with benign lesions (such as fibroadenomas) or with anatomic structures (such as lymph nodes). In particular, lymph nodes can be easily recognised because they exhibit a rich hilar vascularisation, also if small sized. Therefore, power Doppler, when used in addition to B-mode US, may reinforce the benign or malignant suspicion of a solid mass and can help to improve BI-RADS assessment category.

Assessment of lesion vascularity is recommended but is not considered mandatory in the BI-RADS US lexicon [70] that includes three descriptor choices ((a) absent, (b) internal vascularity, (c) vessels in rim). In the US section of the new BI-RADS fifth edition [70], the special category has been expanded with the additional terms of arteriovenous malformations and Mondor disease.

In addition to all potential uses above described, the application of colour Doppler may be helpful during breast interventional procedures, in order to avoid hitting great vascular structures with the risk of bleeding and obscuring the target lesion (particularly frequent in lesions of small dimensions or located deep in the breast). Besides, a biopsy marker clip may create a twinkle artefact, best appreciable with colour Doppler.

18.2.5 US Elastography

US elastography has the ability to measure tissue stiffness, in a similar manner of palpation during physical examination. Two types of elastography are available today: strain and shear wave.

Strain elastography requires gentle compression with US probes that results in a tissue displacement (or strain), usually inversely correlated with tissue stiffness [77]. This technique provides qualitative information, in a colour-scale image, although the strain ratio, comparing the strain of lesion to the surrounding breast tissue, can be calculated [78, 79]. Stiff malignant masses usually exhibit higher strain ratio in comparison with benign lesions.

In shear-wave elastography, the US probe generates transient, automatic pulses that induce transverse waves in the tissue. The US system measures the speed of these waves, which travel faster in hard tissue compared with soft tissue [80]. A quantitative information, represented by the tissue elasticity and measured in KPa or m/s, can be calculated.

Therefore, some parameters obtained with elastographic analysis such as strain ratio, shape, homogeneity and maximum lesion stiffness (Fig. 18.18) can enrich the conventional sonographic features, improving specificity in the diagnosis of breast lesions.

On elastography, malignant lesions typically appear more irregular, heterogeneous and larger compared with greyscale B-mode examination [81, 82]. Moreover, although malignant lesions exhibit maximum stiffness greater than 80–100 KPa [82, 83], a variability among lesions and among elastography techniques may exist [82].

Some papers found that high stiffness, measured at shear-wave elastography, is highly correlated with more aggressive behaviour tumours, including high-nuclear-grade, large-sized lesions and early lymphatic and vascular invasion [84].

Despite these initial promising results about usefulness of elastography in clinical practice, some limitations exist such as differences between the two methods (strain and shear wave) and among different US machines and the presence of inter- and intra-observer variability, affected by degree and method of compression, although shear-wave technique seems to be less operator dependent [85]. In addition, one must be aware that elastographic assessment is less accurate in lesions deeper than 2 cm and that soft cancers or hard benign lesions may exist.

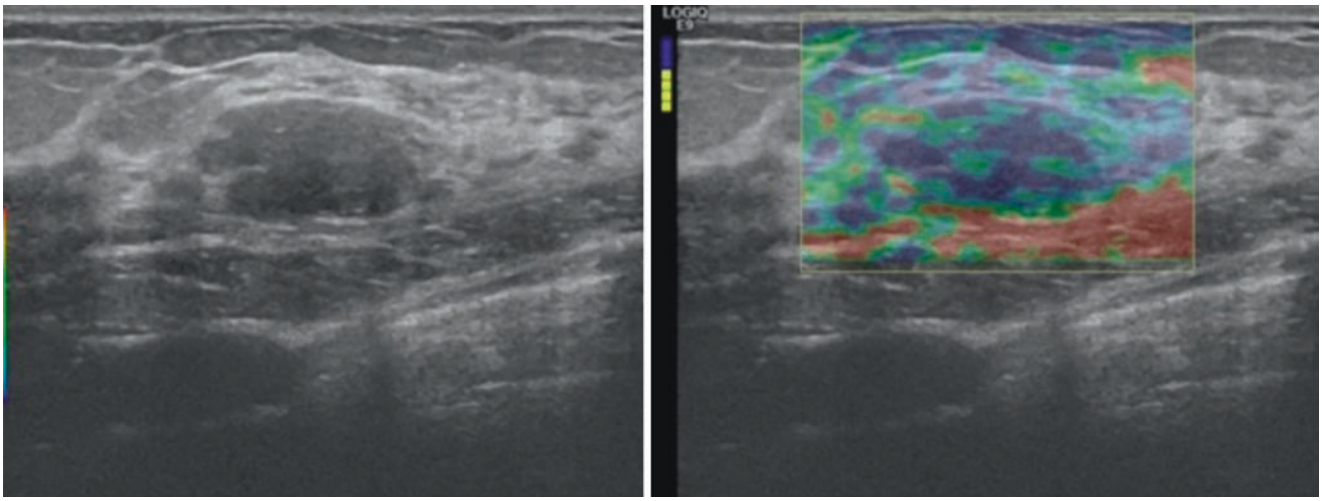


Fig. 18.18 Elastography. Hypoechoic circumscribed lesion (fibroadenoma) that is predominantly elastic, depicting the typical mosaic pattern of *green* and *blue* (BI-RADS US 2) on elastography (*right image*)

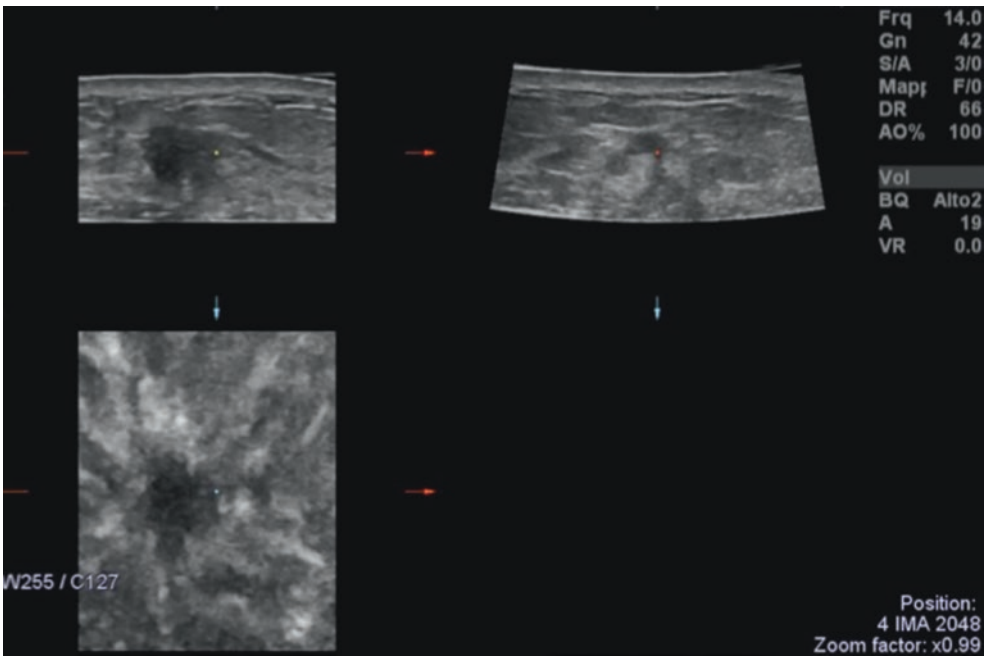


Fig. 18.19 3D ultrasound. Invasive ductal carcinoma grade 1. Images of 3D US are presented in three planes ("multiplanar display mode"). The coronal plane allows a better evaluation of tumour margins and distortion type of growth pattern, typically associated with malignant lesions

18.2.6 Advances in US Technology

New technical developments such as 3D ultrasound, dedicated CAD (computer-aided diagnosis) and automated whole-breast ultrasound (ABUS) are promising methods suitable for the future clinical practice [65, 72, 86].

18.2.7 Three-Dimensional (3D) Ultrasound

3D ultrasound has been recently developed, and high-resolution linear 3D transducers are available in new US machines for a new multidimensional breast imaging. 3D US technology, with a single pass of the ultrasound beam, allows the acquisition of a volume data set, from which the static 3D information will be reconstructed. In 3D US, reconstructed 3D sonographic images are displayed in a "multiplanar display mode" allowing the representation of breast lesions and of surrounding breast tissue in three spatial planes (coronal, sagittal, and transverse plane) [86, 87] (Fig. 18.19). The system allows to navigate through the entire volume, performing parallel movements through the image slices in the three orthogonal planes.

Compared to standard 2D US, 3D images provide a more accurate assessment of tumoural margins and of surrounding breast tissue; moreover, the multiplanar representation is available for a double reading.

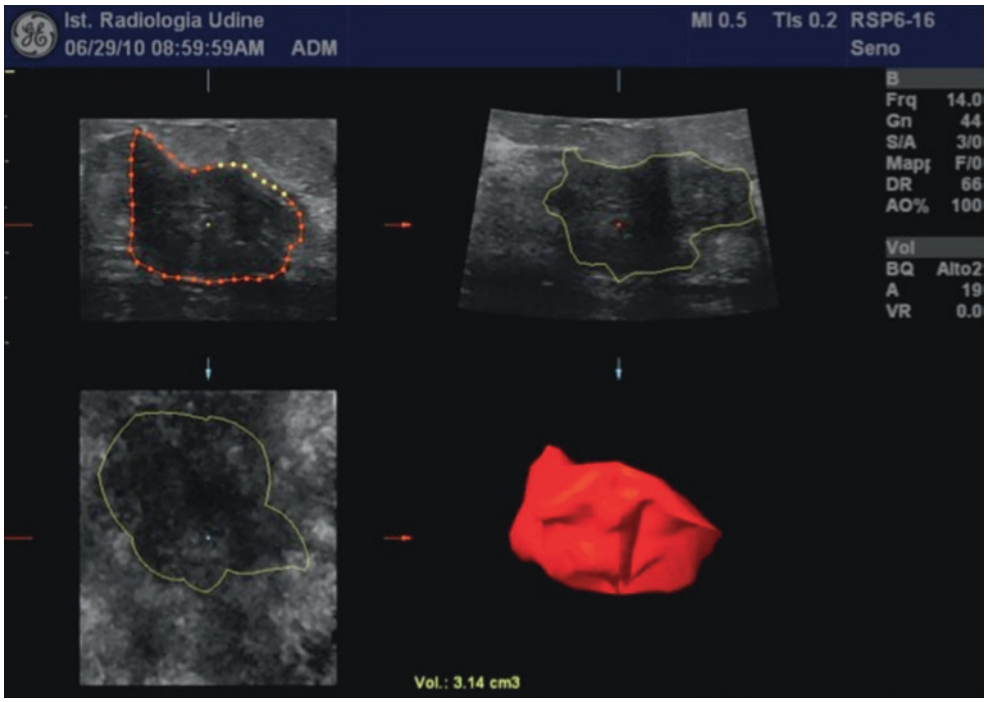


Fig. 18.20 3D Ultrasound. Invasive ductal carcinoma grade 2 with intraductal component, with lesion's volume calculation. 3D US can easily obtain calculation of tumour volume (cm^3) by the VOCAL software ("virtual organ computer-aided analysis")

In particular, Rotten [87], by using 3D US, described two main peritumoural tissue patterns, particularly visible in the coronal plane, corresponding to "compressive pattern", typically associated with benign lesions, and "converging or stellate pattern", associated with malignant lesions. By using these criteria of peritumoural tissue pattern, the authors [87] achieved a 91.4% sensitivity, a 93.8% specificity, an 86.9% positive predictive value and a 96% negative predictive value in the differentiation between malignant and benign lesions.

Moreover, 3D US has a potential role in the assessment of tumoural response to neoadjuvant chemotherapy, offering a precise and reliable volume calculation with VOCAL software ("virtual organ computer-aided analysis") [86] (Fig. 18.20). 3D US can also be used for the volumetric assessment in the preoperative evaluation of breast lesions [88].

A future application of 3D technology should include 3D ultrasound guidance during breast needle biopsies, with the goal of reducing sampling errors due to "partial volume effect", especially with small-sized breast lesions.

18.2.8 Automated Whole-Breast Ultrasound (ABUS)

ABUS is a new technological advance in which breast scanning is performed automatically by using a curved transducer that is larger compared to traditional handheld (HHUS) probe and is similar, in size and shape, to a mammography compression paddle. This automated transducer is placed over the breast using a moderate tissue compression, with patient lying supine on the table, and allows to scan the whole breast automatically. Usually, three acquisitions (AP, lateral and medial) for each breast are needed, which may increase to four or five acquisitions in women with larger breasts. On average, total acquisition time is 15 min, with medium-sized breasts [89].

All imaging data obtained during scanner acquisition are processed and stored on a computer hard drive and finally can be visualised on a standard workstation during reporting and interpretation session. On workstation screen, ABUS images can be displayed in the transverse, coronal or sagittal plane (Fig. 18.21), typically not available with traditional 2D US imaging. In particular, the coronal view is particularly helpful in detecting areas of architectural distortions, which may be difficult to appreciate on standard axial images [90] (Fig. 18.22). ABUS offers also the possibility to visualise real-time images at the time US examination is performed, in a similar manner of HHUS examination.

Automated US offers several advantages over traditional HHUS scanning, such as higher reproducibility, less operator dependence and less physician time for image acquisition. In fact, the physician time required by ABUS includes only time for interpretation, approximately 3 min to read a negative examination, whereas time for image acquisition has been now eliminated [89].

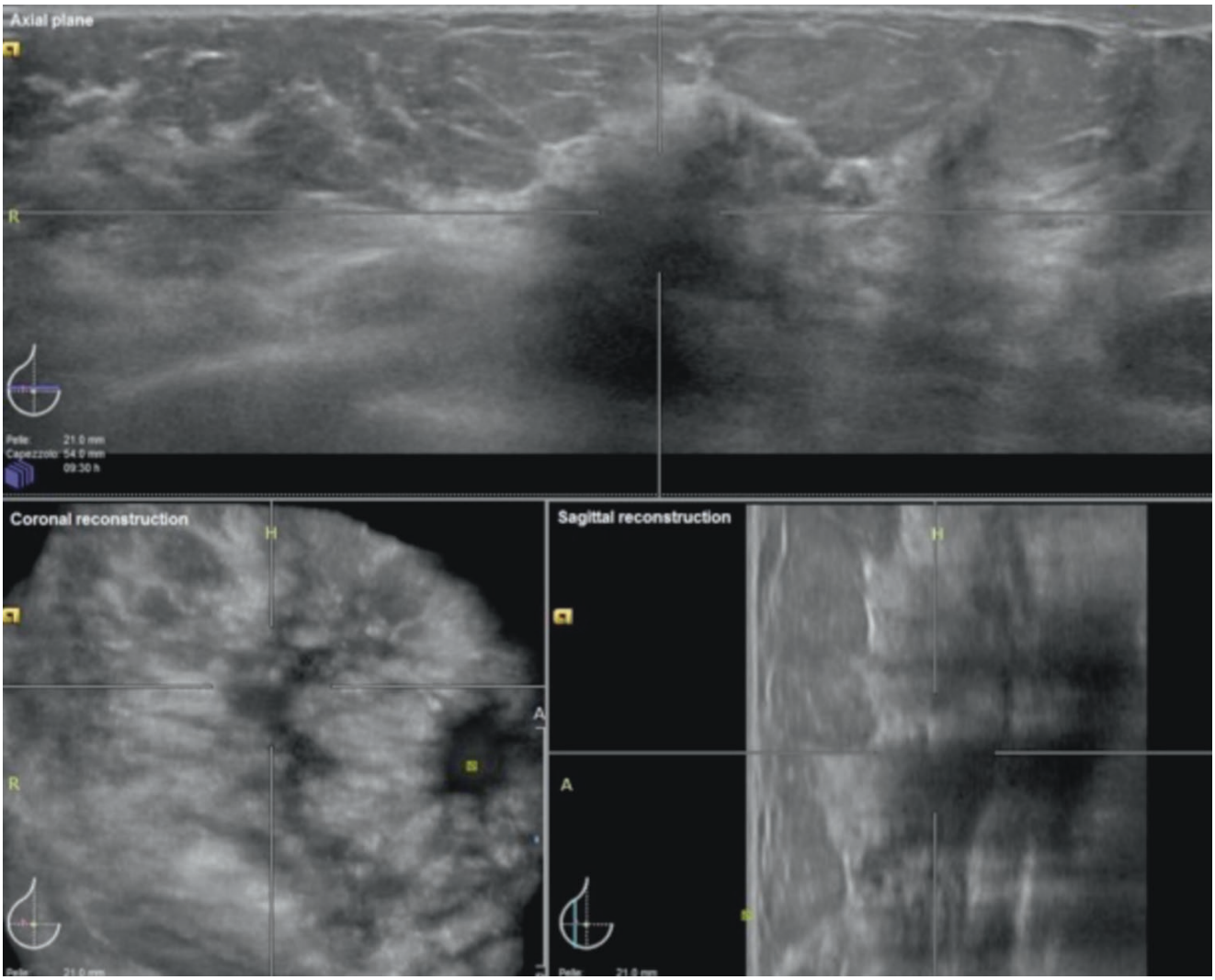


Fig. 18.21 ABUS. Invasive lobular carcinoma. Hypoechoic lesion with indistinct margins and posterior shadowing artefacts (BI-RADS US 5) shown in axial, sagittal and coronal planes

There are some limitations in automated US technology, which are the presence of shadowing artefacts in the subareolar region (that may obscure actual lesions or create unreal findings) and the incomplete assessment of breast tissue, being the axillary region not included in the automatic scanning [89]. Some authors [91] report that ABUS is a promising diagnostic tool with a good interobserver agreement, comparable to that of HHUS, on lesion characterisation and on final category assessment.

One topic of interest is the potential application of ABUS, as a promising screening tool in adjunct to mammography, for examining radiologically dense breasts [89]. In the large observational study of Brem [92], the addition of ABUS to screening mammography in women with dense breast tissue has resulted in an increase of cancer detection rate (1.9 additional cancers per 1000 screened women) but also in an increase of false-positive results. In fact, 552 additional needle biopsies were performed to identify 30 cancers detected with ABUS alone (most of these were invasive clinically important cancers). This high false-positive rate inducing to perform unnecessary biopsies should be however overtook with higher operator experience and higher diagnostic confidence. Some recent works have demonstrated an equivalence in lesion detection [93] and an equivalence or, in some cases, a superiority in lesion characterisation in the comparison between ABUS and HHUS [94, 95].

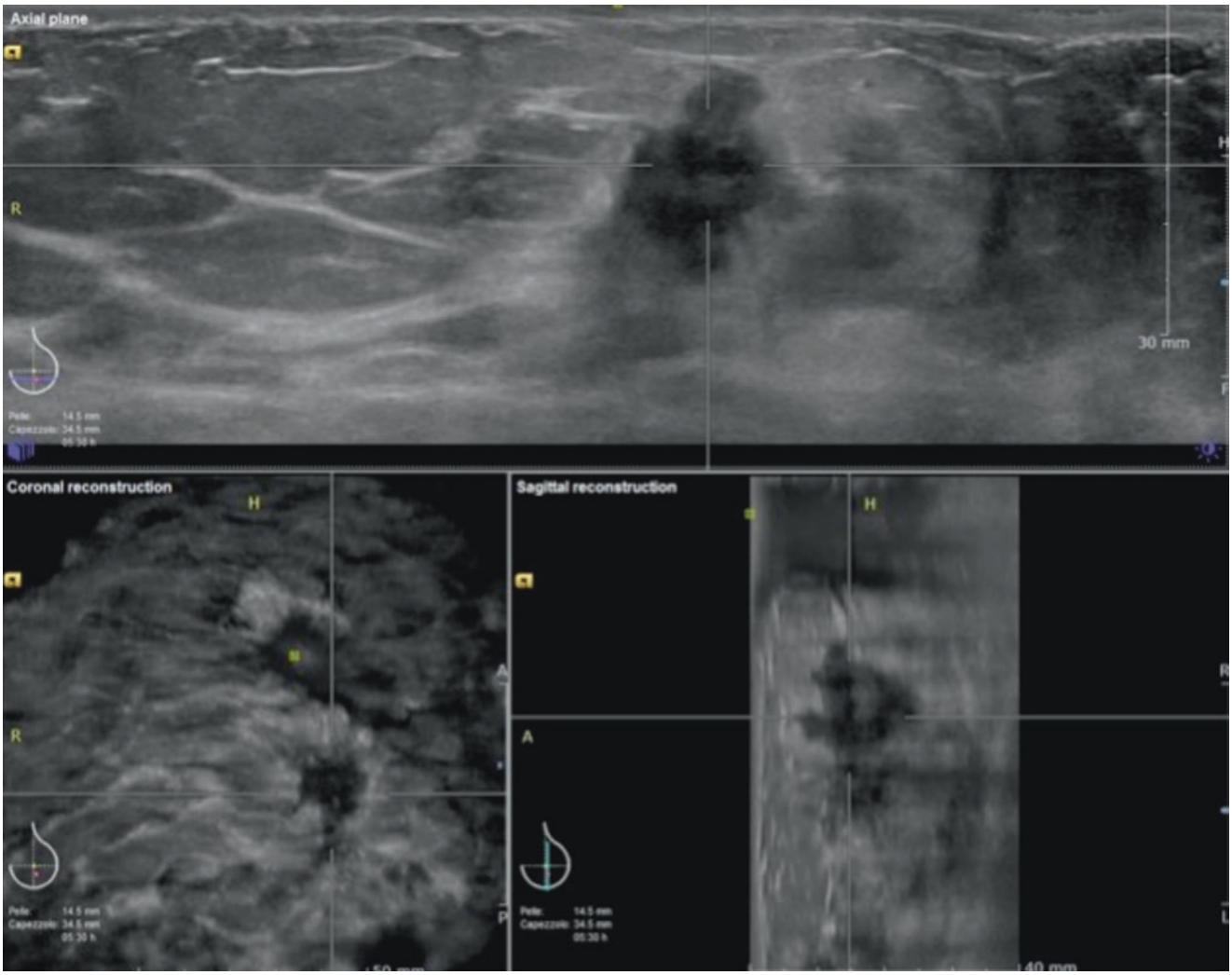


Fig. 18.22 ABUS. Invasive ductal carcinoma. Hypoechoic lesion with vertical growth and irregular margins (BI-RADS US 5) shown in axial, sagittal and coronal planes

18.2.9 Breast Ultrasound in Clinical Practice

Breast ultrasound (US) is the modality of choice for differentiating cystic from solid breast masses, and its primary role is the characterisation of lesions detected at mammography, at MR imaging, or at clinical breast examination [96].

Current indications for breast ultrasound, as recommended by the ACR Practice Guidelines [96], include the evaluation and characterisation of palpable masses or other breast symptoms and the evaluation of abnormalities detected with other imaging modalities, including the role of targeted US after a contrast-enhanced breast MR examination to find an ultrasound correlate. US can also be used as first-line imaging modality for palpable masses in women under 30 years and in lactating and pregnant women and for evaluation of breast implants. In addition, US can be used as guidance for breast biopsy or other interventional procedures, including biopsy guidance of abnormal axillary lymph nodes [96]. The use of bilateral whole-breast US, in women with dense breast tissue, as an adjunct to screening mammography, is a topic of discussion and debate [89, 96].

18.2.10 ACR BI-RADS US

In light of the widespread use and implementation of breast US in clinical practice, a standard lexicon for sonography was initially developed in 2003 by the ACR in order to provide a standardised lexicon for sonographic reporting, to facilitate final category assessment and to validate management recommendations. The correct adherence to BI-RADS

US lexicon can improve differentiation between benign and malignant lesions and potentially reduce the number of unnecessary biopsies [97].

The BI-RADS sonographic categories include size, shape, orientation, margins, echogenicity, lesion boundary, attenuation features, special cases, vascularity, and surrounding tissue [70]. In the last BI-RADS fifth edition [70], new terms have been added to the US lexicon to simplify reporting and to reflect technologic advances (such as the addition of elastography). In particular, a new section, including "elasticity assessment" with three descriptor choices ((a) soft, (b) intermediate, (c) hard), has been added. The ACR recommends the use of these elasticity descriptors instead of the colour scale, not yet standardised.

Some authors report an interobserver variability with the use of BI-RADS US lexicon comparable to that for mammography. Abdullah et al. [98] found a fair interobserver agreement ($k = 0.30$) in the final BI-RADS category, in particular in final BI-RADS 4 a, b, and c subcategories ($k = 0.33$, 0.32 and 0.17, respectively), reflecting difficulties of radiologists to indicate a degree of suspicion. Promising results were obtained by Heinig et al. [99] that reported malignancy rates in BI-RADS US category 3, 4 and 5 similar to those of mammography (1.2%, 17% and 94%, respectively), underlying the usefulness of BI-RADS US descriptors to obtain a final degree of suspicion.

18.2.11 Sonographic Findings of Cystic and Solid Breast Masses

Thanks to its ability to differentiate cystic from solid masses and to state a suspicion degree among solid masses, breast US is usually complementary to mammography in the characterisation of breast lesions, characterisation that sometimes may appear difficult if lesion is small (<5 mm) or located deep in the breast [100, 101].

The traditional sonographic signs used in breast US reporting refer both to the "Stavros' sonographic criteria" [67], which represent a landmark in this context, and to the US descriptors illustrated and validated by the ACR BI-RADS US [70].

1. **Cysts**

   Simple cysts are defined as well-circumscribed, anechoic masses, with posterior acoustic enhancement. Complicated cysts are hypoechoic masses, not vascularised at colour Doppler analysis, that may contain internal echoes or exhibit indistinct margins. Complicated cysts are benign findings, typically associated with low malignancy rate (0–0.08%) [102]; however, when associated with a mammographic correlate or with a palpable mass, they should be classified as BI-RADS 3, and a short follow-up or an US-guided aspiration should be recommended.

   "Complex masses" present a heterogeneous echo pattern with an anechoic (liquid) component and a hypoechoic (solid) vascularised component; sometimes mural nodules, thick walls or irregular internal septations may coexist. In relation to their high malignancy rate (23–31%), these complex masses should be assessed as BI-RADS 4 and should require further characterisation with US-guided needle core biopsy.

   The application of harmonic imaging can improve characterisation of simple cysts (particularly if small sized), allowing the elimination of artefactual internal echoes, whereas the application of elastography is useful in improving the specificity of lesions assessed as BI-RADS 3 or BI-RADS 4a, including complicated cysts, with the result of reducing the need of unnecessary biopsies [82].

2. **Solid Breast Masses**

   (a) Sonographic criteria of benignity

   The benign sonographic features described by Stavros [67] and later confirmed by Hong [103], typically associated with a low risk of malignancy, include ellipsoid or oval shape (negative predictive value, 84%), circumscribed margins with gentle bi- or tri-lobulations (90%), the "wider-than-taller" appearance with parallel orientation (78%), as well as the absence of any malignant features.

   Lesions with these sonographic benign findings are typically fibroadenomas that may be managed with a short-term imaging follow-up, even if the mass is palpable [97, 104].

   However, considerable overlap between benign and malignant US features exists; therefore, a careful correlation with mammography is essential, keeping in mind that an US benign-appearing solid mass requires biopsy if it exhibits any suspicious mammographic features.

   (b) Sonographic criteria of malignancy

   Sonographic findings predictive of malignancy include spiculated (positive predictive value, 86%) or angular (60%) margins, irregular shape (62%), the "taller-than-wider" appearance with antiparallel orientation (69%), posterior acoustic shadowing (52%) and echogenic halo (70%), expression of peritumoural desmoplastic reaction [67, 103]. Other sonographic Stavros' criteria predictive of malignancy include marked hypoechogenicity, microcalcifications, duct extension, branch pattern and the presence of microlobulated margins [67].

   Among US descriptors, Lazarus [40] found a good agreement for lesion orientation, shape and boundary ($k = 0.61$, 0.66 and 0.69, respectively), a moderate agreement for margins and posterior acoustic shadowing ($k = 0.40$, for both) and a fair agreement for lesion echo pattern ($k = 0.29$) and final assessment category ($k = 0.28$).

   In addition to sonographic signs, the value of these criteria in distinguishing benign from malignant solid

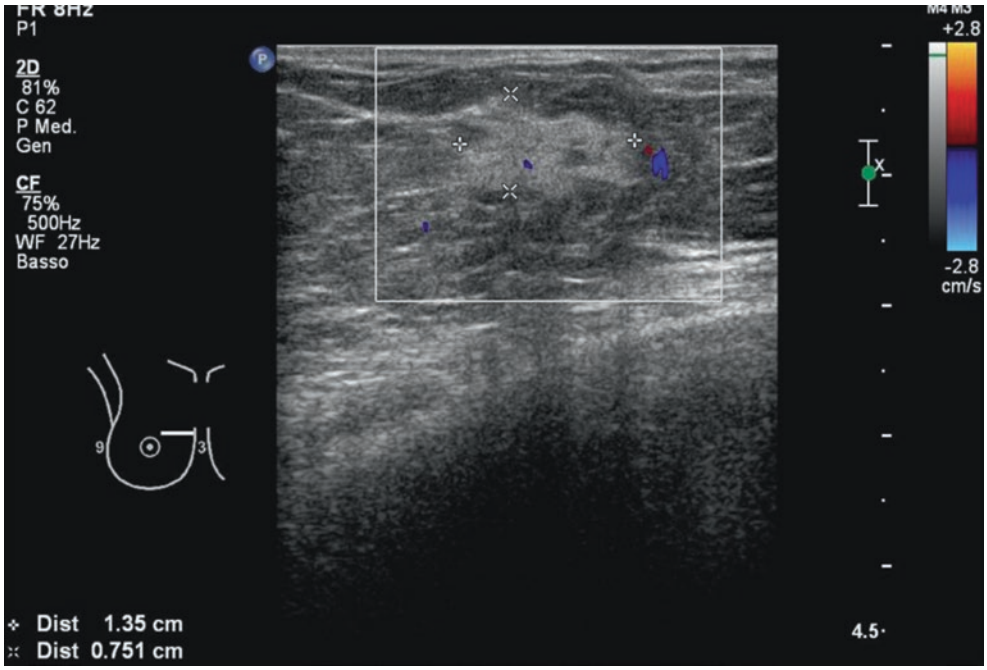


Fig. 18.23 Invasive ductal carcinoma grade 2. Hyperechoic lesion, with irregular morphology and indistinct margins (US signs suspicious for malignancy)—BI-RADS US 4b

lesions is affected also by lesion size, with improving accuracy of breast US when evaluating lesions greater than 7 mm [105].

3. **Hyperechoic Breast Masses**

   Although lesion hyperechogenicity is considered the benign feature with the highest (100%) negative predictive value for malignancy [67, 103, 105], hyperechogenicity at US alone does not exclude malignancy, and uncommon hyperechoic malignancies may exist [106, 107].

   When evaluating a hyperechoic lesion, suspicious sonographic features that should help to avoid misdiagnosis include the presence of focal hypoechoic areas within the hyperechoic lesion, non-parallel orientation, non-circumscribed margins, posterior acoustic shadowing and rich internal vascularisation at colour Doppler examination (Fig. 18.23). In addition, correlation with clinical history and with mammographic appearance should be recommended.

4. **Ductal Carcinoma In Situ and Microcalcifications**

   Ultrasound is considered to have a marginal role in the evaluation of ductal carcinoma in situ (DCIS), in relation to the poor demonstration of microcalcifications, particularly when located in a dense breast parenchyma.

   The advances in US technology with the use of high-frequency transducers (high-resolution sonography) and with improved spatial and contrast resolution allow a better depiction of microcalcifications, particularly when they form large (>10 mm) clusters or when they are located in solid hypoechoic masses, highly suspicious for malignancy.

   US features associated with DCIS usually include hypoechoic masses, intracystic masses, and architectural distortions [108, 109]. In Moon's study [108, 110], a microlobulated mass, with mild hypoechogenicity, ductal extension and normal acoustic transmission, was the most common US finding of DCIS.

   Some studies [108–110] have investigated the potential roles of US in the evaluation of DCIS, including those without calcifications; in particular, (1) US can be used to visualised large (>10 mm) clusters of microcalcifications, suspicious for malignancy; (2) US may be helpful in detecting DCIS without calcifications and in evaluating disease extent; and (3) US may reveal mammographically occult DCIS in dense breasts.

   Another main benefit of US detection of DCIS is to identify the invasive component and to guide interventional procedures that are usually more comfortable and less time-consuming compared with stereotactic breast biopsies.

18.2.12 Conclusions

Breast ultrasound has become an indispensable tool in breast imaging, and, thanks to technological advances, its role is currently not limited to distinguish cystic from solid masses and to characterise solid breast masses but also to identify small malignancies in mammographically dense breasts or to detect abnormalities in patients with breast implants or breast reconstruction.

Re-evaluation of the breast with US targeted upon the site of a suspicious MRI-detected lesion ("second-look US" or "targeted sonography") offers the possibility to identify a correlative lesion on ultrasound so that needle core biopsy

may be obtained using sonographic guidance (as an alternative to MR-guided biopsy).

Finally, US can be used to guide interventional breast procedures (such as needle core biopsies or preoperative needle localisations), with several advantages compared with stereotactic guidance.

18.3 MRI

Christiane K. Kuhl

Abstract Magnetic resonance imaging (MRI) is one of the fastest developing fields in contemporary diagnostic radiology. Within the field of breast MRI, there are currently two major research directions. One direction is to increase the complexity of image acquisition methods in order to further improve our ability to characterise disease, i.e. to distinguish nonproliferative changes, changes with atypias, preinvasive and invasive cancer as well as for improved prognostication, prediction and response assessment, according to the concept of "multiparametric breast MRI". The other direction is to reduce the complexity and facilitate image acquisition as well as interpretation, according to the concept of "abbreviated breast MRI". These two research directions are complemented by the development of new methods for MR-guided biopsy and MR-guided surgery. This chapter reviews the current status of the three development directions.

18.3.1 More Technology for Better Answers: Why We Strive to Improve Breast Cancer Imaging Methods

Today, breast cancer is understood as an entire group of diseases that exhibit significant biological differences in terms of their clinical course and outcome [111]. Former breast cancer classification systems that relied mainly on morphological features have been refined or replaced by classification systems that are determined by the cancer's variable molecular features. Improved knowlegde of these features and their role in cancer progression is not only useful for prognostication, but opens up the possibility to exploit these features for targeted therapies. However, heterogeneity is not only observed between cancers but also within a given cancer [112]. Based on current oestrogen receptor, progesterone receptor or human epidermal growth factor receptor 2 (HER2) and Ki-67 classification systems, it is possible that the majority of individual cells within a given cancer exhibit features that are inconsistent with the assigned overall classification. Such intra-tumoural variations are even more important on a genomic or proteomic level.

Yet the more targeted breast cancer therapies become, the more important will be the issue of intra-tumoural heterogeneity as a source of tumour resistance. The overall clinical course of a given patient may be driven by a relatively small subset of primary tumour cells—the primary cancer and its metastases may thus exhibit different types of receptor status. Accordingly, during the course of disease, targeted therapies may need adjustment to account for secondary mutations and/or compensatory pathways that may yield resistant tumours. This, in turn, has sparked interest in the development of advanced imaging methods that help demonstrate intra-tumoural heterogeneity, depict and quantify response or depict resistance to treatment.

Currently, information on a cancer's biological potential is mainly obtained from histologic, immunohistochemical and molecular biological/genomic processing of cancer tissue that needs to be retrieved from invasive methods, i.e. image-guided biopsy. In patients undergoing novel adaptive neoadjuvant therapies, such biopsies may have to be done even repetitively over the course of treatment in order to monitor treatment-related changes. Research on risk stratification of breast cancer relies on such tissue-based markers that provide information on molecular biology, i.e. genomic and proteomic alterations found in cancer. These techniques have been readily integrated into clinical decision making. A possible shortcoming of this focus on tumour genomics and proteomics is the fact that successful tumour growth does not only depend on a tumour's genomic toolbox but also on its microenvironment, i.e. features of the tissues that host the cancer [113].

Noninvasive, "functional" in vivo imaging tests such as multiparametric MRI refer to the acquisition of information on tissue microstructure and tissue metabolic homeostasis through the use of advanced and increasingly complex MR imaging methods such as higher magnetic fields (3.0–7.0 T systems), improved surface coil technology, improved digitisation of signal transduction, new pulse sequence approaches or hybrid imaging, i.e. a combination of MRI with positron-emission tomography, among many other approaches. Functional imaging helps assess the interaction between a cancer and its microenvironment and the degree to which a cancer is successful in shaping its environment to sustain its growth. Growth pattern, cellular turnover, cellularity, degree and type of vasculature and immune cell infiltrate have an impact not only on clinical behaviour but also on presentation in imaging, i.e. yield the "imaging phenotype" of cancers. Accordingly, functional MR imaging methods provide in vivo imaging biomarkers that correlate with, and, thus, provide surrogate markers of, cancer biology. Such imaging methods therefore promise to provide further independent diagnostic and prognostic information that will add to our understanding of a cancer's ability to grow and metastasize.

Established and new "functional" MRI pulse sequence approaches discussed in the following are diffusion-weighted imaging and its derivatives: diffusion tensor and diffusion kurtosis imaging (DTI and DKI) and "intravoxel incoherent

motion imaging" (IVIM); dynamic contrast-enhanced (DCE) MR imaging including its many varieties and kinetic analyses; blood oxygenation level-dependent imaging (BOLD); and MR spectroscopy (MRS) and spectroscopic imaging (MRSI) of proton (1H) or phosphorus (31P) nuclei—all of which can, in principle, be combined into so-called "multiparametric" breast MRI protocols (mp breast MRI). Moreover, there are completely new pulse sequence approaches such as MRI fingerprinting and chemical saturation transfer or CEST imaging which will probably be used for advanced non-invasive breast cancer phenotyping in the foreseeable future.

Currently, the commonest way to use MRI for breast cancer detection, staging, and classification is by exploiting the angiogenic activity of breast cancers. We identify enhancement, i.e. a signal intensity increase, in images obtained early after intravenous injection of an intravenously administered contrast agent [114, 115]. Tissues that accumulate the injected contrast agent appear bright on so-called T1-weighted post-contrast MR images. To track the enhancement of lesions, clinical breast MRI protocols always consist of a so-called dynamic series. This means that a stack of cross-sectional images is obtained before and then repetitively after the i.v. bolus injection of the contrast agent. Cancers are characterised by fast and strong enhancement that is observable already on early post-contrast images, usually followed by a washout of signal intensity. Benign changes and the normal fibro-glandular tissue exhibit less and usually only slowly progressive enhancement over time. Since angiogenic activity is the main driver of enhancement, all regular clinical breast MR protocols will reflect this activity (Figs. 18.24 and 18.25). Accordingly, even the most basic breast MRI study will contain "functional" information on pathophysiological changes that are implicated in carcinogenesis and metastatic growth [116]. Moreover, MRI depicts these changes by true 3D

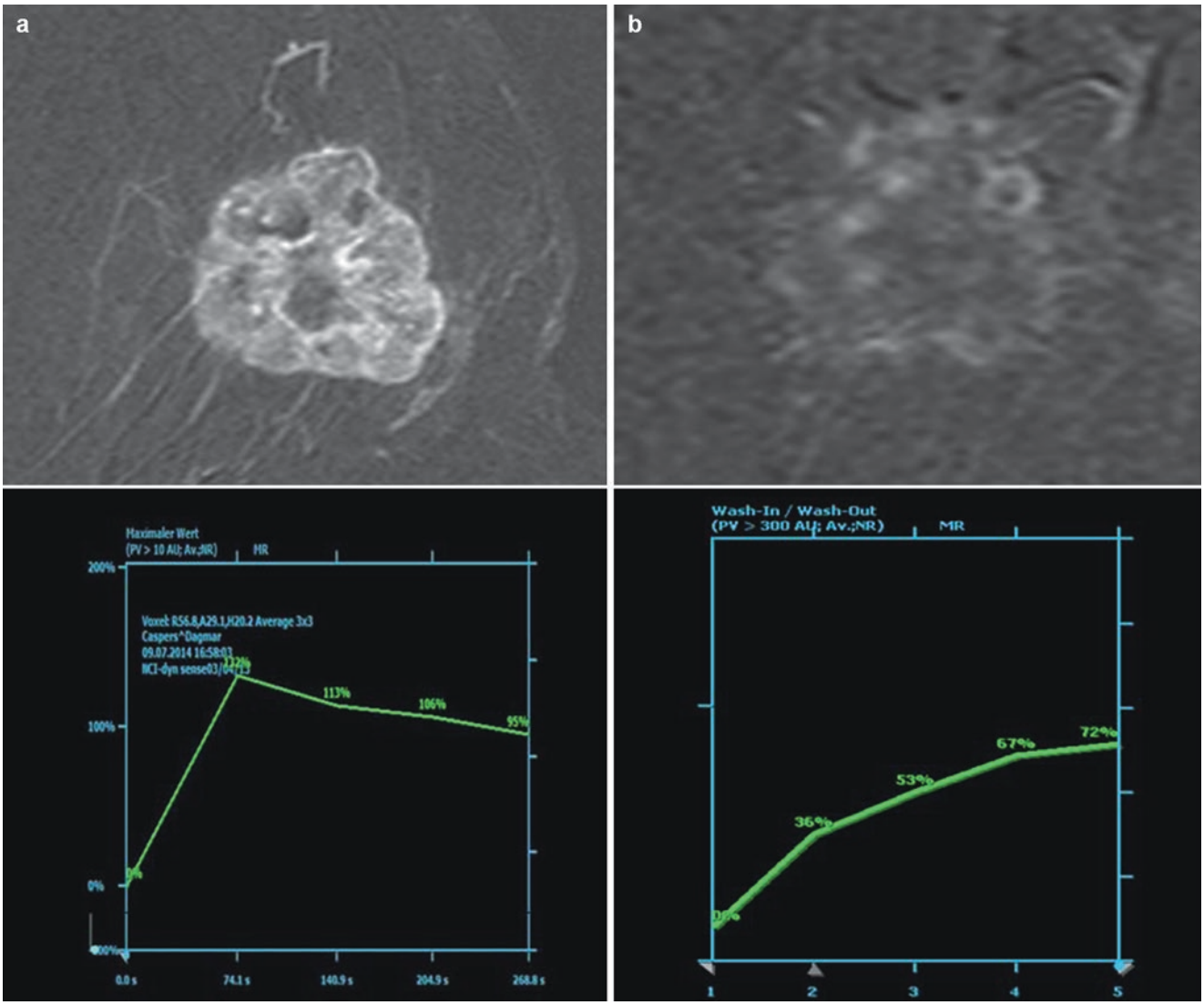


Fig. 18.24 DCE-MRI for response assessment: Note strong and early enhancement with washout time course at baseline and slow enhancement with flattened enhancement curve after the first cycle. Tumour size is still unchanged. (**a**) Before chemotherapy, (**b**) after first cycle of chemotherapy

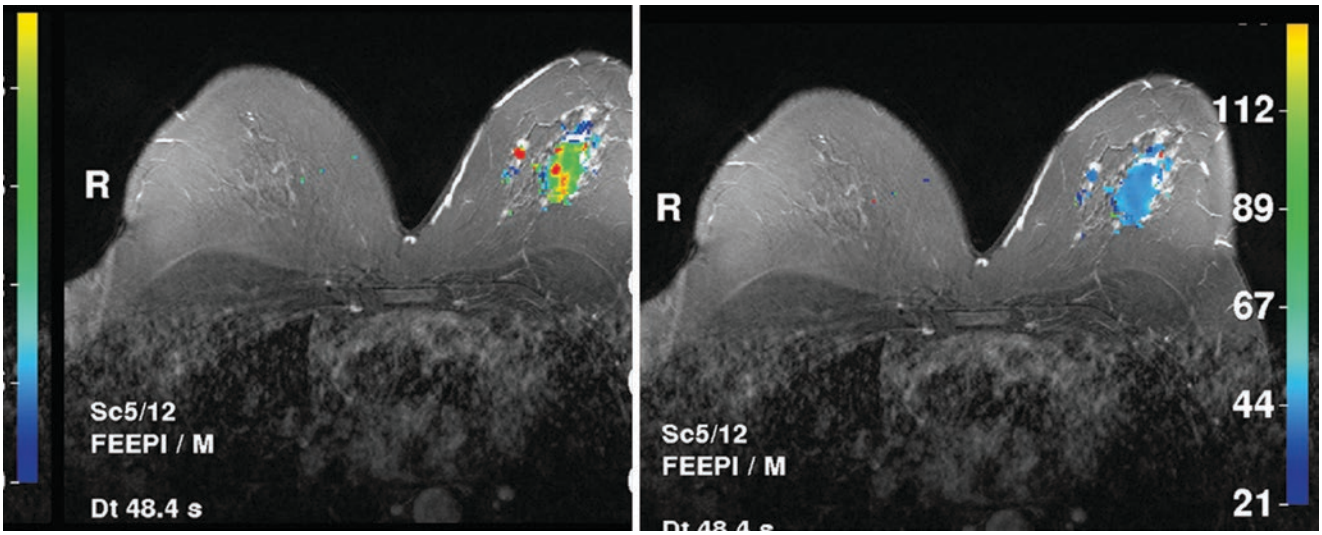


Fig. 18.25 Colour coding can be used to depict within-tumour heterogeneity of enhancement kinetics and the change of enhancement after neoadjuvant chemotherapy. *Left image*, enhancement map of the cancer at baseline, before treatment. *Right image*, enhancement map of the cancer after the first cylce of neoadjuvant chemotherapy. Note that the size of the cancer is unchanged, but the enhancement pattern has changed towards slowly-progressive enhancement

cross-sectional imaging—unlike, e.g. breast tomosynthesis that provides planigraphic images that are not true, but only "quasi" cross-sections—similar to conventional tomography used before the advent of CT. In contrast, breast MRI allows the depiction of tumour margins and internal architecture with very high spatial and contrast resolution. The assessment of such morphological details is usually best possible in images obtained early after contrast injection, i.e. at a time when the signal intensity difference (i.e. the contrast) between the enhancing cancer and the progressively enhancing adjacent fibro-glandular tissue is maximal.

Diffusion weighted imaging (DWI) is based on the Brownian molecular motion of free (interstitial) water. The distance over which an interstitial water molecule can travel depends on its microenvironment. The smaller the interstitial space, and/or the more cell membranes build barriers against free diffusion, the slower will water diffuse, and the shorter is the distance water can travel within a given period of time. The concept of diffusion-weighted imaging is to "label" water molecules and, after a specific waiting time, sample their response. The more diffusion is restricted, the more molecules will stay in place and be able to contribute to the MR signal [117]. Diffusion is restricted in tissues with increased intracellular, and thus, reduced interstitial space, either due to cell swelling, e.g. in the context of hypoxia, or due to increased cellularity of tissue, e.g. in the context of cancer. DWI can thus be used as a noninvasive measure of the cellularity, to serve as surrogate marker of the proliferation fraction of cancer [118]. Tumours with high Ki-67 levels are hypercellular compared with surrounding normal breast tissue, which translates into restricted diffusion of free water molecules on DWI. It has been shown that tissue apparent diffusion coefficient or ADC values correlate with proliferation rates in luminal-B cancers [119, 120]. Another important clinical application of diffusion-weighted imaging is assessing response of breast cancer to neoadjuvant chemotherapy (Fig. 18.26).

Diffusion tensor imaging and diffusion kurtosis imaging investigate not only the mobility of water but also its directionality [121]. Diffusion might be directed, i.e. facilitated in specific directions, and impeded along other directions of tissue, depending on the microstructure of tissue. Accordingly, DTI as well as DKI can be used to demonstrate tumour ultrastructure and infiltrative growth way beyond the resolution of regular structural MR imaging [118, 122]. IVIM is increasingly used as a non-contrast means to depict perfusion of tissue, again on a microstructural, i.e. capillary level [123, 124].

MR spectroscopy is a well-established technology that has been in use for decades for analytical tests in biochemistry. It interrogates noninvasively the (quantitative) distribution of specific metabolites in a given probe. The underlying principle is the fact that the Larmor (resonance) frequency of a given nuclide, usually proton or phosphorus, depends on (a) the respective type of nuclide and on (b) the individual magnetic field in which the nuclide resides. The magnetic field experienced by a given water or phosphorus nucleus will depend on the individual molecular environment of that nuclide, because there will be shielding of the magnetic field by different neighbouring atoms. The individual, specific structure of molecules will thus modulate the magnetic field experienced by a proton or phosphorus nuclide. Thus, nuclei bound in different molecules will be exposed to a slightly different magnetic field. Since there is a direct correlation

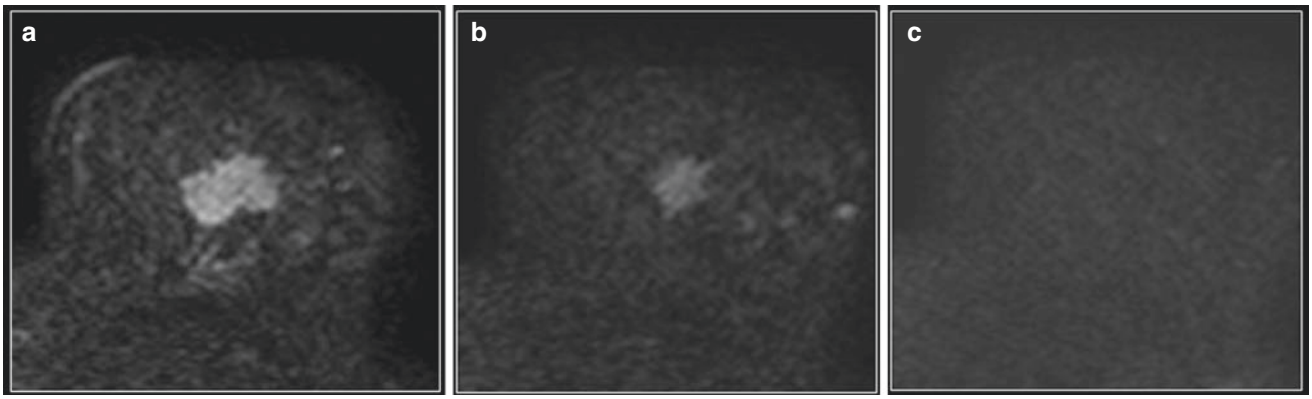


Fig. 18.26 Use of diffusion-weighted imaging for response assessment. (**a**) Baseline, (**b**) Mid-treatment and (**c**) After treatment. Note on (a) the bright signal on DWI at baseline, due to diffusion restriction secondary to the high cellularity of cancer. At mid-treatment (**b**), the cytotoxic effects lead to reduced cellularity of the tumor, such that diffusion of free water is improved, and the DWI signal drops. At conclusion of treatment, the diffusion of free water is back to normal; the DWI signal is isointense to normal tissue. Histology confirmed complete pathological response (pCR)

between the magnetic field and a proton's resonance frequency, protons or nuclei located in different molecules will exhibit slightly different resonance frequencies. Magnetic resonance spectroscopy displays the distribution of resonance frequencies and, thus, of different metabolites or molecules in a given probe. For in vivo MR spectroscopy of the breast, most experiences exist with proton MRS. Compared with ex vivo biochemistry measurements, many effects such as field inhomogeneity and low SNR lead to the fact that the spectral resolution that is attainable in patients is not as high as in ex vivo MRS of biochemical probes. Thus, the individual resonances of individual molecules are broadened; the observable spectral peaks usually comprise several different resonances (and, thus, metabolites). For 1H MR spectroscopy of the breast [125], detection of protons bound in choline compounds has been found to be clinically useful [126]. The detectable choline peak represents proton bound in free choline, in phosphocholine and in glycerophosphocholine. Other constituents will be phosphoethanolamine and myo-inositol. Cellular turnover, either anabolic or catabolic, may increase the contribution of phosphocholine to the observable choline [127, 128]. 1H MRS has been shown to help discriminate breast cancer from benign enhancing lesions and as a prognostic marker to assess cellular (i.e. membrane) turnover. Especially rapidly growing tumours will lead to a detectable choline peak (tCho). Moreover, MR spectroscopy is useful to demonstrate early response to neoadjuvant chemotherapy. Reduction of total choline helps distinguish between responders and nonresponders after two treatment cycles (PPV and NPV of 89% and 100%); with current technology, it is, however, limited to the analysis of larger tumours, i.e. locally advanced breast cancer [127–130].

Tumours need to maintain growth by increasing their local supply with oxygen and nutrients. This is achieved by releasing peptides like VEGF that induce local angiogenesis. Angiogenesis leads to a fundamental change of a tumour's microvascular architecture, with sprouting of existing vessels as well as development of de novo formed vessels, usually with fenestrated vessel wall linings that go along with increased vessel permeability. The increased metabolic turnover leads to an increased amount of toxic waste products that are removed through dilated drainage veins. The increased perfusion leads to the well-known strong and early enhancement in DCE-MRI, and the increased permeability, together with the efficient venous drainage, causes the washout time course that is characteristic for breast cancer [131]. It has been shown that DCE-derived enhancement kinetics correlate with estrogen receptor status, HER2 status, nuclear grade/Ki-67 and EGFR expression.

The increased permeability leads to leakage of larger molecules such as proteins from the intravascular to the interstitial space, which will increase the oncotic (colloid osmotic) pressure within the cancer—a fact that drags water from the intravascular into the interstitial space and thus increases the interstitial water volume fraction. This, in turn, will correlate with a cancer's signal in T2-weighted imaging. If angiogenesis fails, or is insufficient to reach the innermost cell layers of a cancer, then hypoxia will occur, again detectable through the tumour's internal architecture of enhancement in DCE-MRI (rim enhancement) or through BOLD contrast MRI [132].

The abovementioned functional MR imaging methods are thus used to depict tissue features on a microstructural level. The respective pulse sequences are usually associated with borderline signal to noise ratio (SNR). To improve SNR, the use of higher magnetic fields such as 3.0 T or, more recently, even 7.0 T promises an even more accurate and extensive assessment of tumour biology [127, 133, 134].

Functional imaging methods can be used for classification of enhancing lesions seen in breast MRI, i.e. for the further differentiation of benign, high-risk and malignant lesions in breast MRI. The combination of high-resolution cross-sectional

morphological information, enhancement kinetics and lesion's signal in T2-weighted images and in diffusion-weighted images yields a high specificity and positive predictive value of contemporary breast MRI protocols. Even the most basic, 1.5 T dynamic contrast-enhanced breast MRI protocols are inherently "multiparametric" compared with, e.g. mammography or DBT. The diagnostic accuracy achieved with such protocol is sufficient to be used for so-called problem-solving.

Accordingly, and in contrast to currently held beliefs, we have recently shown that breast MRI can indeed be used definitely to settle screen-detected mammographic or ultrasound findings and thus help avoid unnecessary biopsies [135].

More importantly, functional imaging methods provide additional, independent diagnostic information that adds to our understanding of a cancer's ability to sustain its growth and/or its propensity to metastasise. Multiparametric MRI techniques can therefore be used to investigate a tumour's aggressiveness and its biologic and prognostic importance or its response to systemic treatment [120, 123, 136–140].

In the neoadjuvant situation, the local breast cancer serves as an in vivo marker to rate the efficacy of systemic treatment protocols. Since even regular, clinical breast MRI studies provide functional information on tissue perfusion, it is possible to depict response to treatment earlier than what is achievable by imaging methods that rely on tumour size estimates only such as radiographic imaging methods (digital mammography, digital breast tomosynthesis) or ultrasound-based methods (Figs. 18.24, 18.25, 18.26, 18.27, 18.28, 18.29 and 18.30; Table 18.3) [141–146]. Results on the use

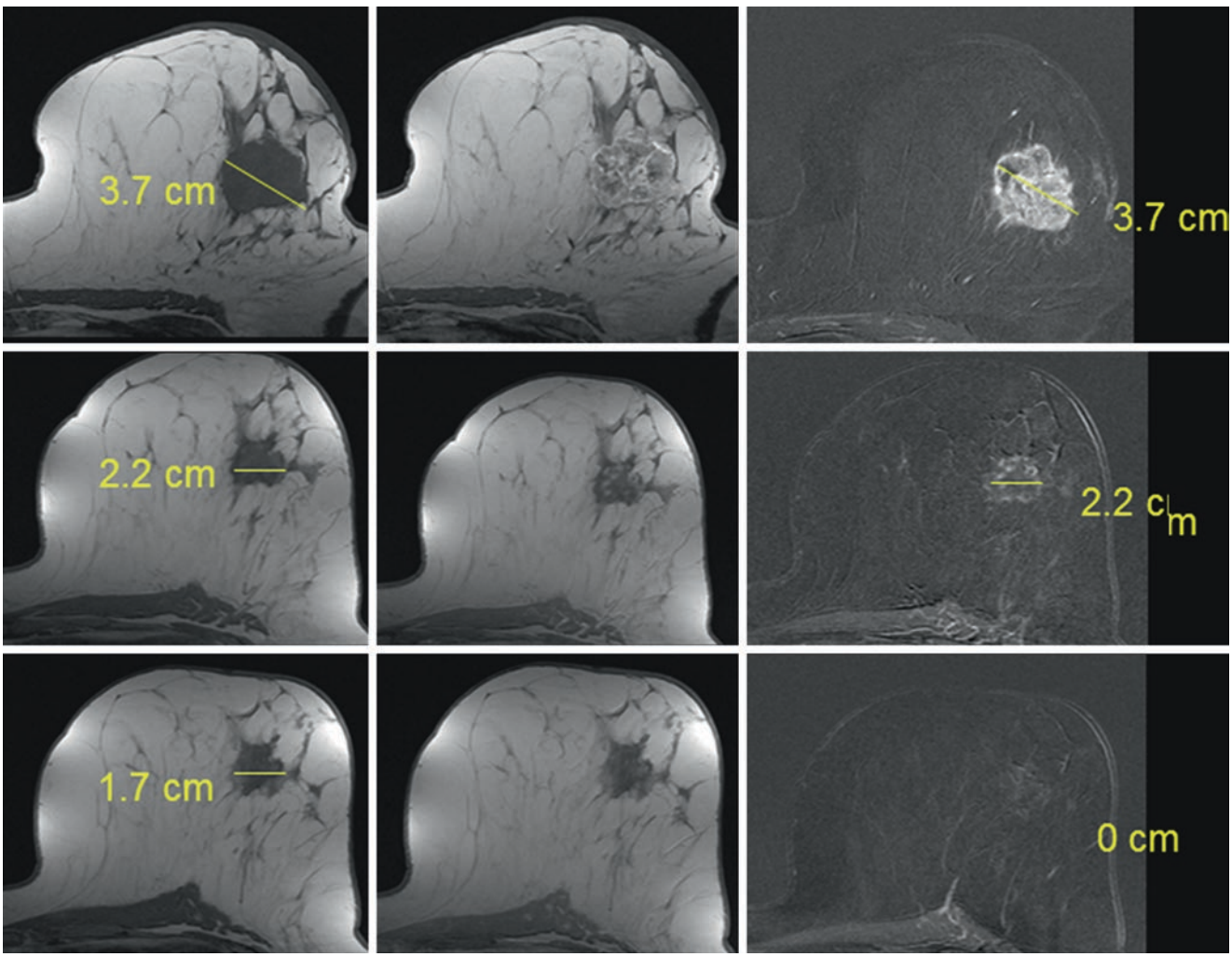


Fig. 18.27 Utility of DCE-MRI vs. structural breast imaging. Patient with triple-negative breast cancer. Incomplete response was suggested based on breast ultrasound and mammography, with residual diameter of 1.7 cm. This residual mass is also visible on pre-contrast T1-weighted imaging of her breast MRI study (*right column*). However, after contrast injection, DCE-MRI (*middle column*, post-contrast source images; *left column*, corresponding subtracted images) reveals absence of enhancement in the remaining tumour, suggesting presence of scar formation. Complete pathologic response with fibrotic tumour remnants without vital tumour cells was found at histology

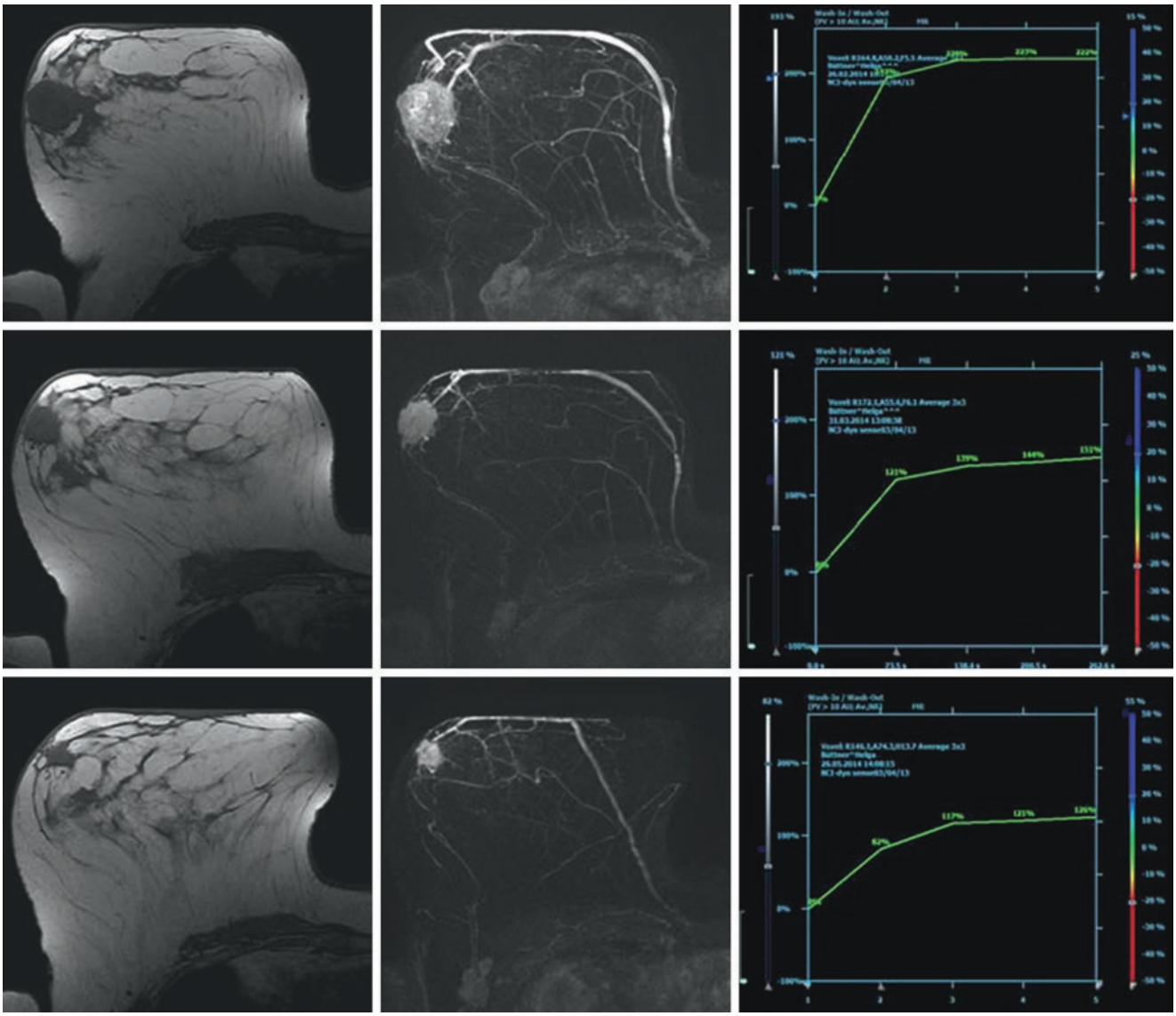


Fig. 18.28 Patient with residual disease after neoadjuvant chemotherapy. Minimal residual mass visible but with strong enhancement. Histology confirmed presence of a 9 mm residual vital tumour. *Upper row*, MRI before chemotherapy. *Middle row*, MRI at mid-treatment. *Bottom row*, MRI after conclusion of neoadjuvant chemotherapy

of more advanced functional imaging methods or "multiparametric MRI" for this purpose are emerging [141]. For instance, diffusion-weighted imaging and DKI are candidate methods to further improve assessment of response; an increase in mean tumour ADC (apparent diffusion coefficient) of over 20% from baseline is predictive of response in triple-negative and HER2-enriched cancers (Fig. 18.30) [143, 146, 147]. Similar results have been obtained for the detection of changes as early as a couple of days after even a single administration of chemotherapy by proton spectroscopy [128, 140, 146–151].

Another task in the neoadjuvant setting is to identify residual disease. Since there can be scar tissue formation at the site of the previous tumour, this is a difficult task with imaging methods that rely on depiction of structure alone. Since regular DCE-MRI, but even more so advanced multiparametric MRI provides information beyond structure, it is much more accurate than, e.g. mammography or ultrasound for this purpose. Marinovich et al. provided an excellent review on the evidence of using regular clinical breast MRI for predicting response to neoadjuvant chemotherapy. They reported on 13 different studies, comprising 2549 patients who underwent DCE-MRI before and after one to two cycles of neoadjuvant treatment. They found that the accuracy for prediction of response is highest in studies that evaluated enhancement kinetics and found a mean accuracy of 88% [142].

The next step now is to use the plethora of imaging features provided by multiparametric breast MRI to describe "MR imaging phenotypes" of breast cancers. Modern methods of machine learning ("deep learning") can then be used

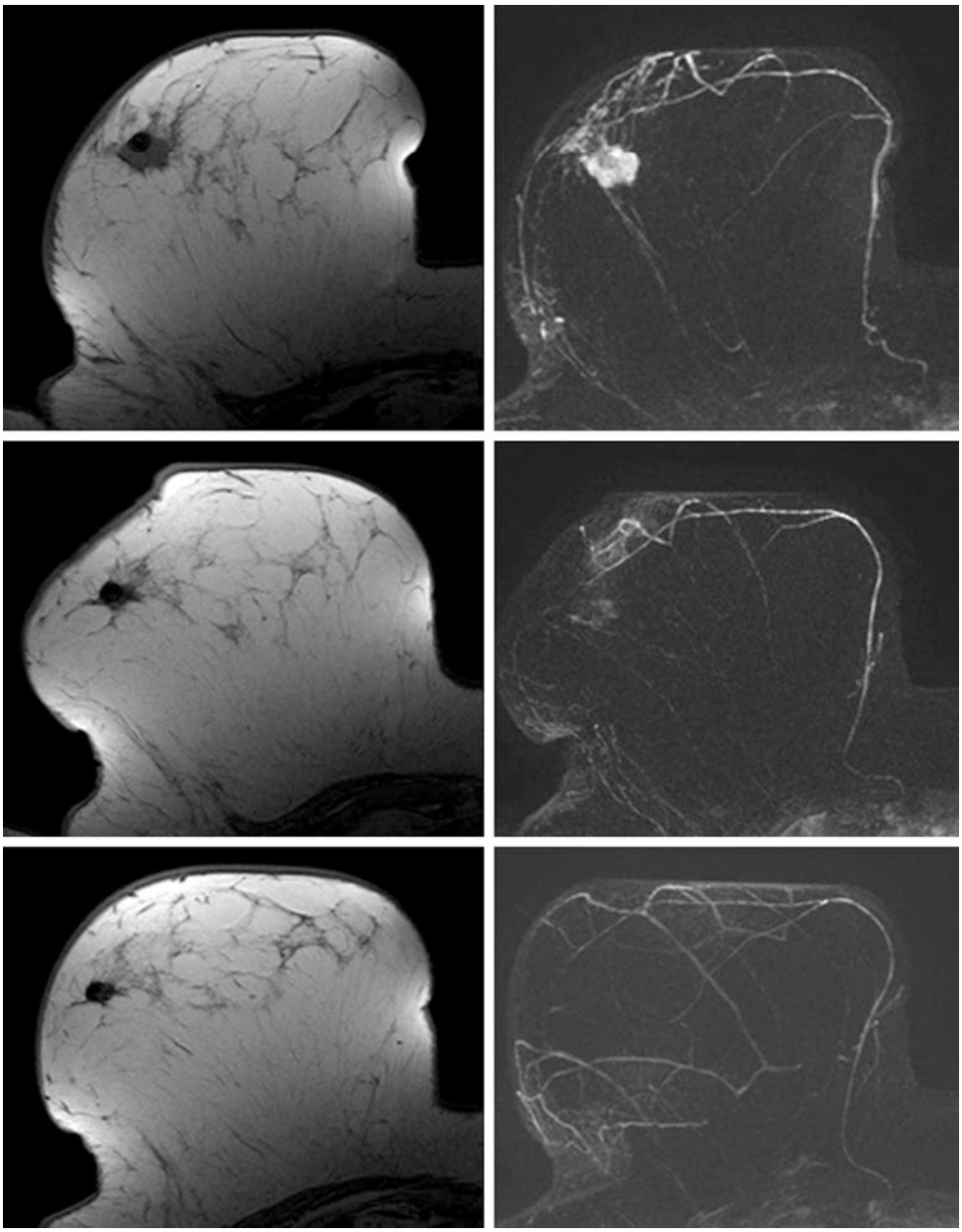


Fig. 18.29 Prediction of pCR based on DCE-MRI. Pre-contrast images (*right column*) to provide structural information and post-contrast subtracted images (*left column*) to demonstrate enhancement. Already at mid-treatment (*middle row*), there is almost complete loss of enhancement at the site of the cancer. Absence of enhancement was noted at completion of neoadjuvant treatment (*lower row*). Pathological complete response was found. The focal black spot at the site of the cancer corresponds to the clip inserted after US-guided biopsy

to correlate different MR imaging phenotypes with patient outcomes, similar to the way genomic typing has been correlated with outcomes to establish their prognostic utility. It is to be expected that such "radiomics" will be helpful to amend the predictive and prognostic information derived from genomic and proteomic studies.

18.3.2 Keep It Simple and Short: Abbreviated Breast MRI for Cancer Screening

Although there has been a decline in breast cancer mortality over the last two decades, breast cancer continues to represent the first (Europe) or second (USA) leading cause of cancer death in the female population. Notably, several decades of mammographic screening programmes have not changed this situation. Since there is a close correlation between disease stage (i.e. the size and stage distribution of cancer) at the time of diagnosis and ultimate survival of an individual woman, the persistently high mortality rates indicate that there is room and need for improved methods of early diagnosis of breast cancer. Interval cancer rate, i.e. the number of cancers that occurs in women who did participate in mammographic screening, but are not diagnosed by mammography, compared to the number of cancers that are mammography detected, ranges between 30% and 50%. These interval cancers are associated with adverse biologic profiles and poor prognosis compared to screen-detected cancers. Accordingly, the current scientific evidence suggests that mammographic screening is associated with a

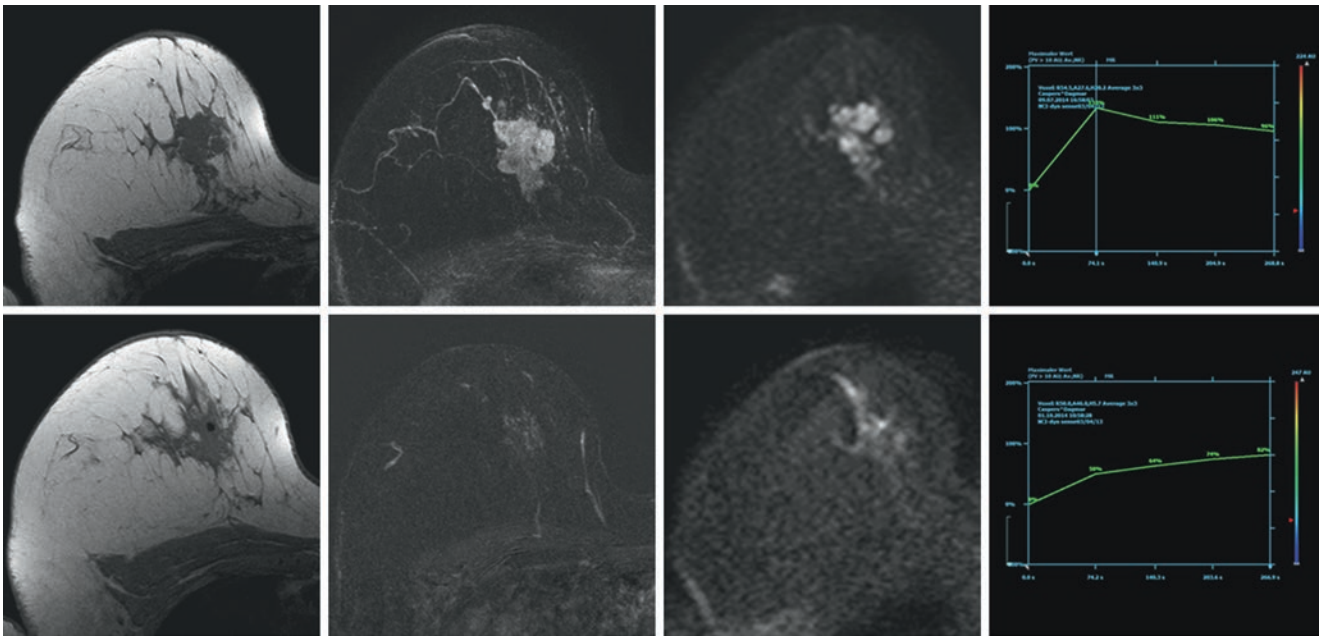


Fig. 18.30 Utility of diffusion-weighted imaging to complement DCE-MRI for response assessment. *Upper row*, baseline study. *Lower row*, study after completion of neoadjuvant treatment. *Left column*, pre-contrast T1-weighted images. *Middle left column*, contrast-enhanced subtracted images. *Middle right column*, corresponding DWI at $b = 800$. *Right column*, corresponding time/signal intensity curves. Note that there is still questionable enhancement at the site of the index cancer. DWI supports the diagnosis of incomplete response or presence of residual disease, with still visible diffusion restriction. Note the clip that marks the centre of the index cancer in the images after treatment

Table 18.3 Published meta-analyses on using breast MRI for predicting pCR after neoadjuvant chemotherapy

Author	Journal	No. of studies	No. of patients	Sensitivity for pCR	Specificity for pCR
Yuan et al. [144]	Am J Radiology 2010	25	1213	DCE-MRI: 63% (55—70%)	DCE-MRI: 91% (91–92%)
Wu et al. [3]	Breast Cancer Res Treat 2012	34	1932	DCE-MRI: 68% (57–77%) DWI: 93% (82–97%)	DCE-MRI: 91% (87–94%) DWI: 82% (70–90%)
Marinovich et al. [142]	J Nat Cancer Institute 2013	44	2549	DCE-MRI: 89–92%	DCE-MRI: 83%
Lobbes et al.	Insights Imaging 2014	35	2359	Correlation between residual disease on pathology and MRI: 0.698	Overall accuracy: 88%

significant underdiagnosis of breast cancer, that is to say, mammography fails to pick up cancers that are prognostically relevant [152, 153].

In addition, mammographic screening has been associated with overdiagnosis of breast cancer. Overdiagnosis relates to the fact that cancers that are picked up by mammography may be biologically unimportant. Some breast cancers can exhibit rapid growth and become life-threatening and difficult to treat; others are relatively slowly growing. Some cancers, especially many screen-detected DCIS, may indeed prove self-limiting and will not progress to a life-threatening disease, even if left undiagnosed and thus left untreated [154, 155].

An important reason for overdiagnosis is a well-established effect referred to as "length-time bias". Women whose cancers were screen detected, i.e. women whose cancers were mammography detectable, enjoy a better prognosis than women whose cancers were not screen detected, i.e. mammographically occult. In short, mammography-detectable cancers are associated with a better prognosis than cancers that are occult on mammography. This, in turn, is attributable to the fact that for diagnosis of breast cancer, radiographic breast imaging methods (mammography or also tomosynthesis) rely on the depiction of regressive changes such as calcifications, architectural distortions and fibrosis, i.e. pathophysiological processes that are associated

with slowed growth, with tissue hypoxia or with frank tissue necrosis. Overdiagnosis is length-time bias put to an extreme. Overdiagnosis causes a huge financial burden to the society; most importantly, however, it leads to unnecessary anxieties and morbidity in women who are stigmatised as "cancer patient" and treated as such, without benefit for the involved women.

In summary, mammographic screening is associated with both significant underdiagnosis of prognostically important breast cancer and overdiagnosis of prognostically unimportant, i.e. self-limiting breast cancer, or rather pseudo-disease.

Magnetic resonance imaging (MRI) has been used to diagnose breast cancer over the last three decades; it is in use for screening for 15 years. Over and over again, it has been shown that contrast-enhanced MRI is by far the most accurate imaging method to diagnose invasive as well as intraductal breast cancer and primary as well as recurrent cancer, irrespective of breast density [9–13, 156–162]. In view of the important discussion around overdiagnosis, the most important feature that makes breast MRI an attractive screening tool is its sensitivity profile. The sensitivity of breast MRI increases in parallel with the prognostic importance of breast cancer. It is exceedingly high in rapidly growing, heavily perfused disease, and it is desirably lower than that of mammography for low-grade DCIS. Thus, MRI is associated with a "reverse length-time bias". This, together with the fact that MRI works without ionising radiation, makes MRI the most promising screening tool that is currently available.

A screening trial completed in our department suggests that MRI screening is not only beneficial in high-risk women but also in average-risk women. The gradient between the diagnostic sensitivities of MRI, compared to that of mammography or even the combined use of mammography and ultrasound, appears similar, more or less independent of the respective lifetime risk of women [163]. We found that if MRI is used in women at average risk, the interval cancer rate drops down to zero—which compares to an interval cancer rate, i.e. missed cancer rate, of around 30–50% for quality-assured European mammographic screening programmes. Since interval cancer rates are the single most important driver of mortality rates, there is good reason to assume that using MRI instead of mammography for breast cancer screening would allow a substantial further reduction of breast cancer mortality. Also the positive predictive value—a major driver of costs associated with screening—is similar for mammographic and MRI screening, suggesting that the previously held belief of the low specificity of MRI has been overcome with growing clinical experience with screening breast MRI.

The single main reason why MRI is not used on a broader scale is the cost associated with this method. Therefore, in 2014, our group inaugurated the concept of "abbreviated breast MRI" (AB-MRI) [164]. We were able to demonstrate that MRI, due to is high-contrast images, can be completed within a magnet, i.e. examination time of only 3 min, and, most importantly, can be read by radiologists within a few seconds. The superior diagnostic accuracy and cancer yield that is afforded by MRI was preserved also for these abbreviated protocols. A group of 443 women with mildly increased risk of breast cancer and with normal screening mammograms and normal screening ultrasound underwent 606 screening MRI studies with abbreviated and full protocol. Abbreviated MRI detected a total of 11 cancers that had been occult on the respective digital mammograms and screening ultrasound studies, for an additional cancer yield of 18.2 per 1000 (Fig. 18.31a). The examination time of the abbreviated protocol had been 3 min, and the average radiologist reading time to establish absence of breast cancer had been 2.8 s. For comparison, even batch reading of a screening mammogram

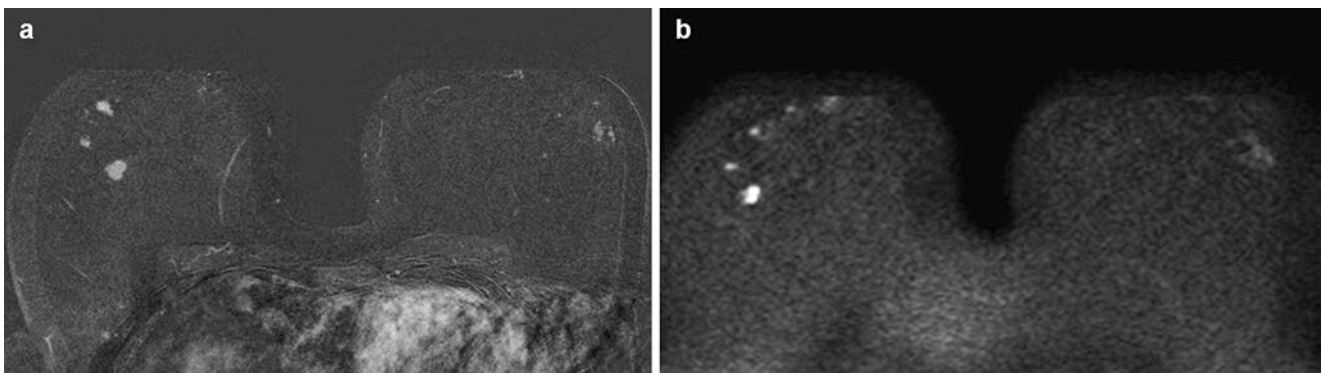


Fig. 18.31 Utility of abbreviated breast MRI, and un-enhanced diffusion-weighted imaging in a 52-year old woman at average risk undergoing MRI screening; her mammogram was normal. (**a**) First post-contrast subtracted or FAST image, generated by subtracting the image obtained within 60 s after contrast injection from the pre-contrast image, for a total acquisition time of 2 minutes. Note the multifocal breast cancer visible in the right breast and absence of cancer in the left breast. Note that similar information is provided by the diffusion-weighted image, which was obtained prior to contrast injection

usually takes over 60 s, and the acquisition of the four views that constitute a screening mammogram takes well over 4 min. For screening ultrasound, the reading and/or scanning time takes about 20 min on average, i.e. takes far more radiologist time and is thus far more expensive—and far less sensitive or specific than breast MRI. Abbreviated breast MRI has sparked great interest in the broader use of breast MRI for breast cancer screening. Several studies have meanwhile been published that confirm the high accuracy of abbreviated protocols. The ECOG/ACRIN (Eastern Cooperative Oncology Group/American College of Radiology Imaging Network) has launched a multicentre prospective randomised trial (EA1141) that will investigate not only the cancer yield but also the type of cancers detected by abbreviated breast MRI compared with contemporary, digital breast tomosynthesis [165]. Several countries have started their own abbreviated breast MRI screening studies.

Moreover, there is evidence to suggest that abbreviated protocols could even work without injection of contrast agents. By using diffusion-weighted imaging with background suppression (DWIBS), cancers are detectable and correctly classifiable with an image acquisition time of only a couple of minutes and very short radiologist reading times (Fig. 18.31b) [166–168].

To fully exploit this for improved breast cancer screening, it would be important to develop dedicated breast MRI systems that are optimised for imaging the breast and optimised to support the fast throughput that is required for broader screening applications. Very similar to the development of dedicated X-ray machines for imaging the breast (i.e. mammography systems) back in the 1940s, this could be done for MRI scanners.

18.3.3 Advances in MR-Guided Interventions

Breast MRI studies are done to obtain information on presence and extent of breast cancer beyond what is available through radiographic or ultrasound imaging. If this is achieved and breast cancer is identified or suspected on MR imaging, it is important to offer noninvasive means to retrieve tissue from the suspected area. No breast radiologist would ever offer a breast imaging service without being able to also offer mammography and ultrasound-guided needle localisation and (vacuum) biopsy—but it seems to be quite popular to run a breast MRI service without such interventional capacities. This is increasingly inacceptable, regarding the fact that equipment for MR-guided vacuum biopsy and MR-guided needle localisation and bracketing is commercially available and has been commercially available for almost two decades now. Accordingly, the American College of Radiology requires availability of such equipment or proof of an established collaboration with sites that offer these interventions in order to receive an accreditation for breast [169].

Recently, we inaugurated the concept of MR-guided vacuum-assisted large-volume biopsy (VALB). For this intervention, we collect larger amounts of tissue than what is usually retrieved during mammography or MR-guided vacuum biopsy procedures, i.e. between 24 and 60 samples with a 9G needle. Such MR-guided VALB was done on a cohort of 1414 consecutive MR-only visible lesions with a false-negative rate, i.e. a rate of missed lesions, of 0.3% (4/1414), all four discovered immediately after the procedure due to an obvious radiologic-pathologic mismatch. The cohort consisted of target lesions with an average size of 9 mm for mass enhancement, and 23 mm for non-mass enhancement, found in small to very large breasts and located in all locations, including far dorsal, far medial, far lateral or immediate retroareolar locations. The results suggest that MR-guided VALB helps avoid previously reported causes of technical failures of MR-guided biopsies. Moreover, we could show that MR-guided VALB procedures are very well tolerated, with a complication rate (major complications) of 0/1414 (Fig. 18.32) [170].

Reoperation rates tend to be high for breast cancer surgery, especially if a high rate of breast conservation is attempted. A recent editorial published in the New England Journal of Medicine was entitled *Re-excision—The Other Breast Cancer Epidemic* [171]. There are numerous reports that consistently show that MRI is more accurate than mammography or ultrasound for demonstrating the extent of a given cancer. If MRI is done for this purpose, however, it is of utmost importance to help the surgeon translate the imaging information into the operating theatre. Only if this is achieved, it is possible to actually exploit the diagnostic advantage afforded by MRI compared with mammography or ultrasound. If one strives to reduce the number of surgical procedures, it is of course important to use nonoperative, nonsurgical biopsy methods to obtain histologic proof of presumed additional disease components prior to surgery. We have used MRI, followed by MR-guided vacuum biopsy, and MR-guided bracketing of the disease extent if needed (Fig. 18.33), in a cohort of 600 women with biopsy-proven breast cancer. We found that this led to a positive margin rates below 4%, which was achieved at a very high breast conservation rate of close to 90%. These data suggest that, if MRI is combined with contemporary methods of MR-guided biopsy, as well as methods to guide surgery, the improved diagnostic information provided by MRI do indeed translate into improved surgical results, low reoperation rates and very low mastectomy rates [172].

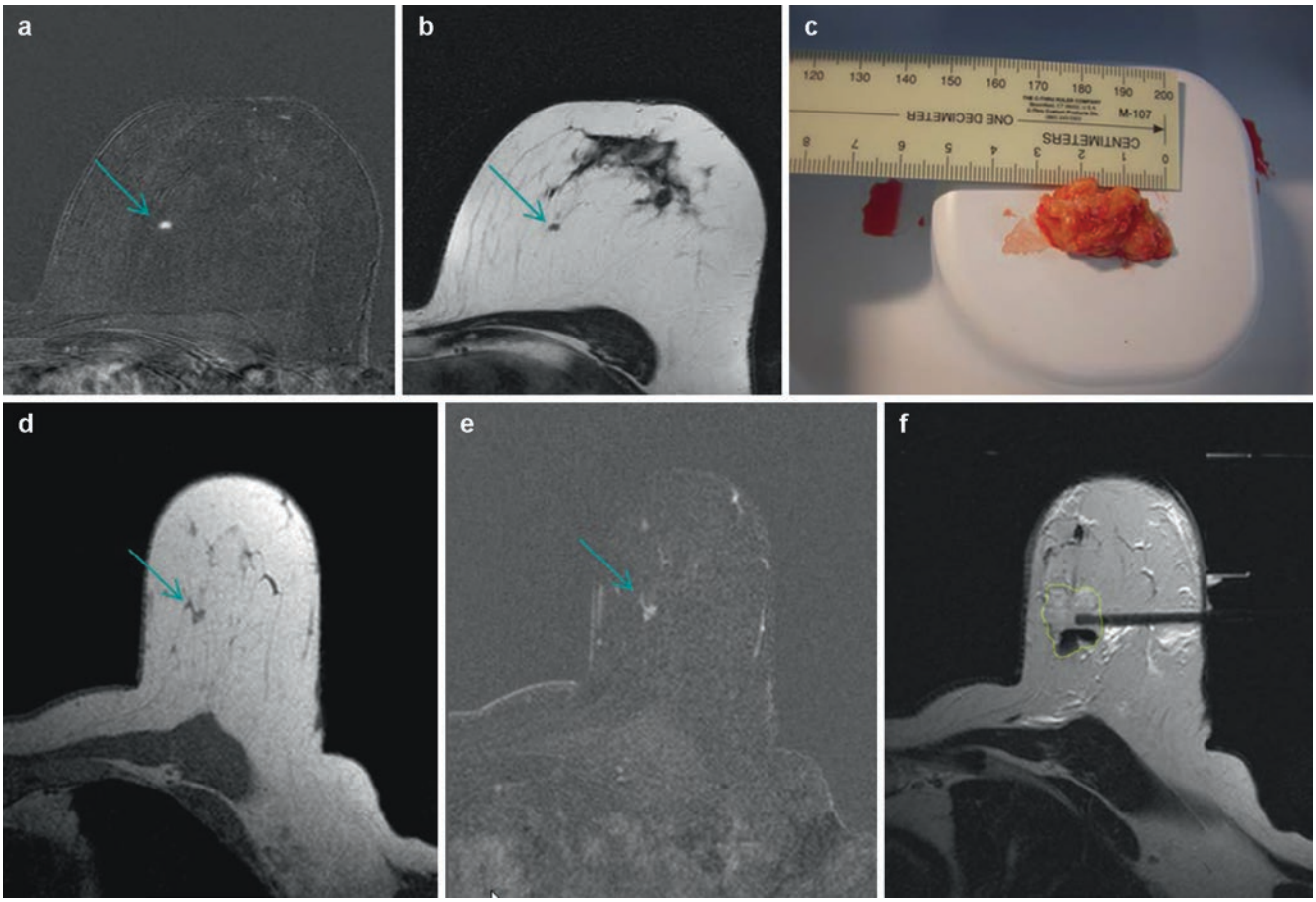


Fig. 18.32 MR-guided vacuum-assisted large-volume breast biopsy (VALB). (**a**) FAST image that highlights a small enhancing lesion in the upper inner quadrant, 4 mm in longest diameter. (**b**) Corresponding T2-weighted structural image. (**c**) T1-weighted image prior to contrast injection during the intervention. (**d**) Corresponding post-contrast subtracted image reveals the target lesion. (**e**) T2-weighted image after completion of vacuum-assisted large-volume breast biopsy, with the biopsy needle still in place. The *yellow line* encircles the biopsy cavity that includes an *air bubble* (*black* signal inside the cavity). Note that the biopsy cavity includes the entire lesion, plus safety margin. (**f**) Removed tissue volume during MR-guided VALB. Histology confirmed pT1a; subsequent surgery proved absence of residual tumor

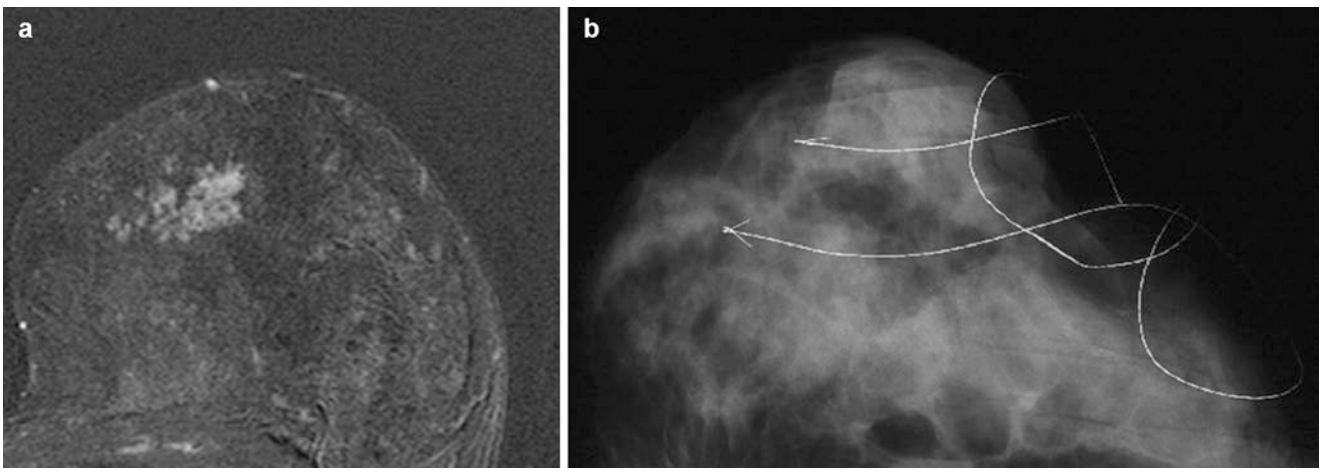


Fig. 18.33 MR-guided surgery. (**a**) Patient with MRI screening detected non-mass enhancement suggestive of DCIS. MR-guided biopsy (not shown) confirmed presence of high-grade DCIS. Patient underwent MR-guided bracketing of the two poles of the enhancing segment. (**b**) Mammogram obtained after MR-guided bracketing displays the guide wire position in the breast and absence of any correlate of the DCIS on mammography. MR-guided surgery was done and revealed a 3 cm high-grade DCIS, resected with free margins (R0)

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Nuclear Medicine in the Clinical Management (ROLL, SNB, and PET)

19

Giovanni Paganelli, Federica Matteucci, and Laura Gilardi

19.1 The Radio-Guided Occult Lesion Localization (ROLL)

G. Paganelli and F. Matteucci

19.1.1 Introduction

The most important goal of modern surgical oncology is to utilize the less aggressive methods while maintaining radicalism. The evolution of imaging techniques and the option of using screening tests more and more reliable and effective have permitted an increasingly early diagnosis, identifying malignant lesions of ever smaller dimensions. This is particularly common in the case of breast cancer, where clinically occult lesions are diagnosed with increasing frequency, now represents approximately 25–35% of all breast cancers diagnosed in developed countries [1, 2].

Numerous techniques have been used to localize non-palpable lesions, but there is no international consensus about which technique combines the best conditions and should be considered the gold standard.

The wire-guided localization (WGL) is currently the location method most commonly used in many centers. [3–5]. This technique, however, has some disadvantages, due to various factors: in general there is a difficulty of the wire positioning in the dense breasts; a dislocation of the wire once it has been positioned is rather frequent [6] resulting in excessive volumes of tissue removed. Finally, thread breaks or dislocations can lead to complications and inconvenience for patients [7, 8].

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In 1996 at the European Institute of Oncology in Milan, the ROLL technique (ROLL: radio-guided occult lesion localization) was introduced for the first time, gaining popularity because of the many benefits associated with the ability to center with greater precision the lesion within the surgical sample, thus reducing the volume of the breast removed with consequent best aesthetic results [9–11].

Moreover, feasibility studies have shown that the technique is simple, rapid, and precise; furthermore, the method is intuitive, and necessary skills are easily acquired [12].

Recently a cost-benefit analysis published by Postma et al. [13] in a randomized controlled trial (RCT) found no difference considering both economic costs associated with morbidity and reoperation.

Retrospective and prospective studies performed on very large populations have shown that the ROLL procedure allows the surgeon to make a precise removal of non-palpable lesions of the breast thus overcoming most of the disadvantages of the previous techniques.

19.1.2 Technique

The day before surgery, a dose of human serum albumin macroaggregates (MAA), with particles of diameter between 10 and 150 μm, labeled with 7–10 MBq of Tc-99m is injected in correspondence of the central portion of the lesion. The tracer preparation mode as well as the quality controls on the dose must be carried out by following the instructions from the product package insert.

In the presence of microcalcifications, opacity, or distortions highlighted with mammography but not visible in ultrasound, it is necessary to use the mammographic apparatus incorporating a computerized stereotactic system (Fig. 19.1a), while in the presence of lesions visible by ultrasound, the radiopharmaceutical is injected under guide ultrasound (Fig. 19.1b).

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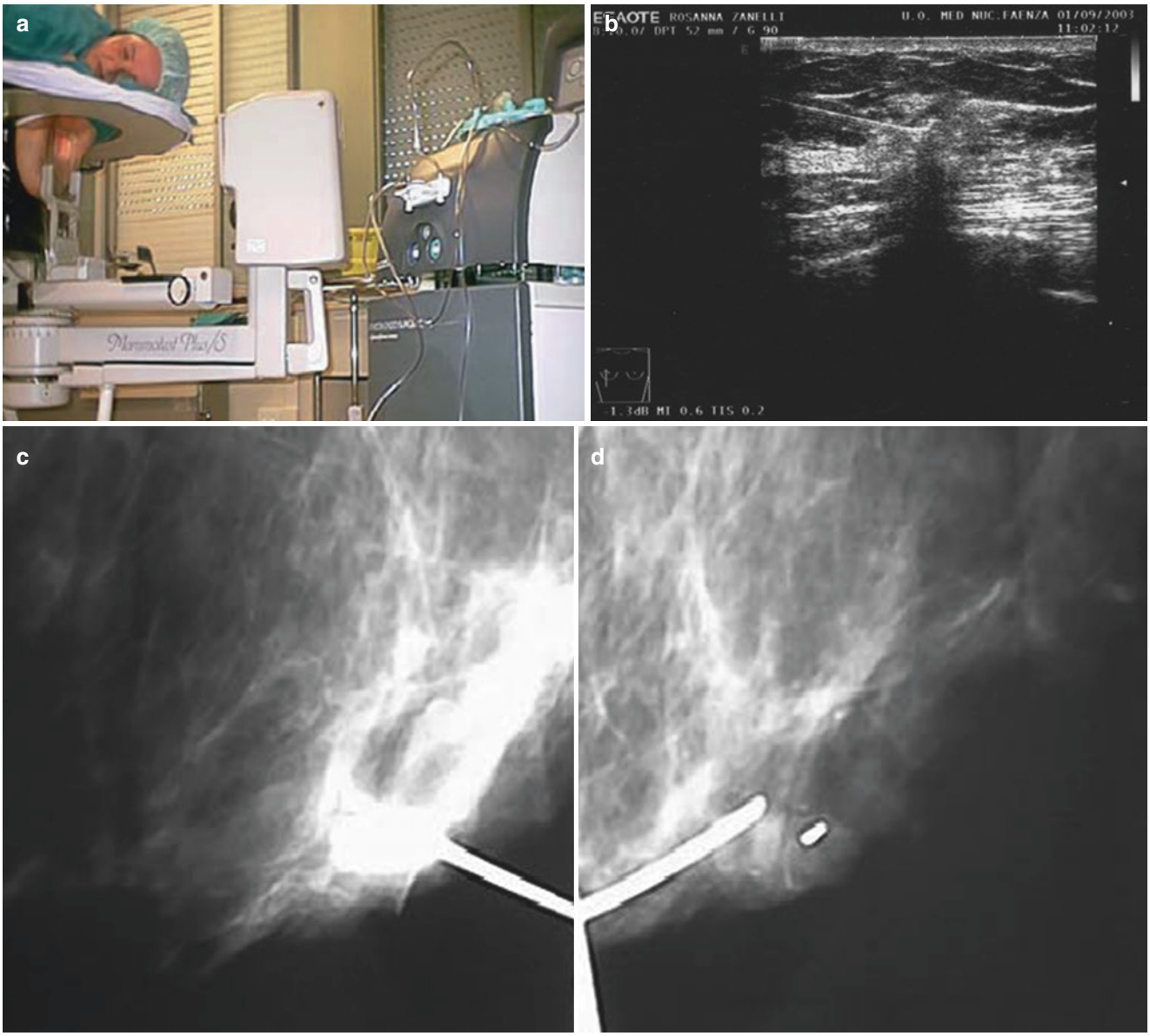

Fig. 19.1 Injection of 99mTc-MAA under (**a**, **c**, **d**) stereotactic or (**b**) ultrasound guidance

For lesions visible by both methods, the tracer is preferentially injected under ultrasound guidance, which allows for greater precision in locating the lesion. Once injected of the dose, the site of inoculation is indicated on the skin with a skin marker pen, so as to serve as a guide both for the next scan and that for surgery.

The control of the exact site of inoculation can be performed by introducing a minimum amount of radiopaque solution within the lesion, immediately after the radioactive substance, and performing a radiological control (Fig. 19.2).

19.1.3 Scintigraphy

Scintigraphic images are acquired usually about 10 min after injection of the tracer in the front and lateral projection. A cobalt-57 source (point source or flexible wire) is used to outline the contour of the breast during the acquisition and to facilitate the viewing of the site of inoculation.

Scanned images must highlight the presence of a focal spot of the tracer accumulation with well-defined margins, without contamination of the skin or spreading in the neighboring locations to the lesion (Fig. 19.3).

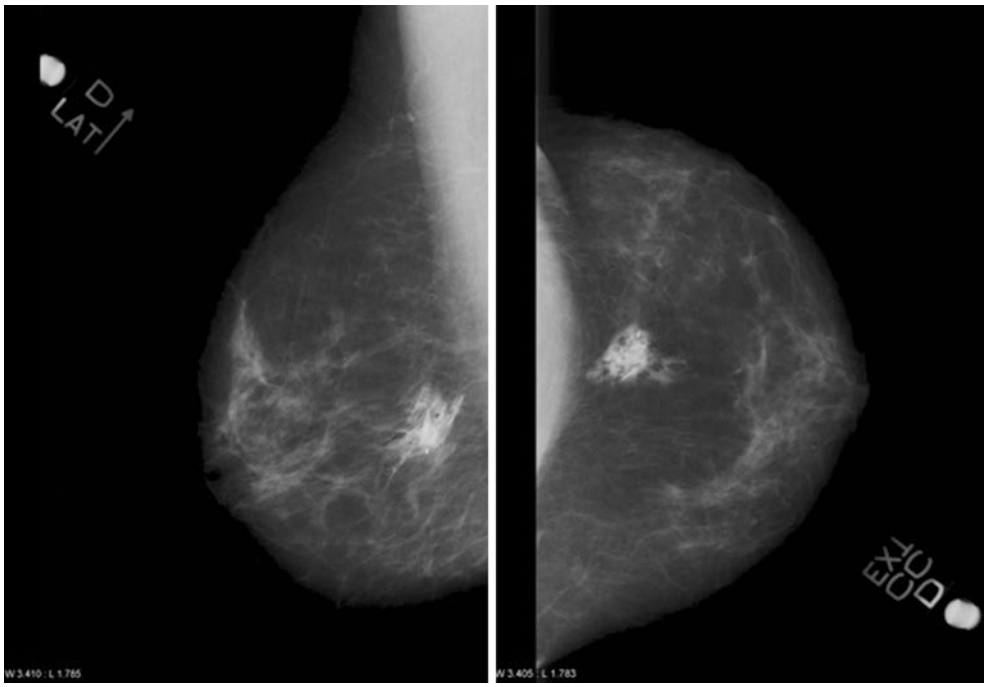

Fig. 19.2 Radiological verification of the exact site of inoculation, immediately after MAA and radiopaque solutions' administration

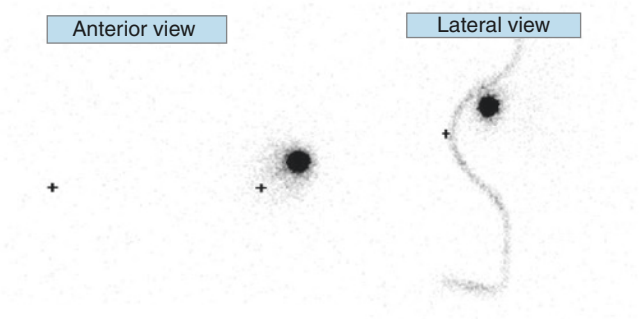

Fig. 19.3 Scintigraphic images of the right breast in frontal and lateral projection performed after injection of 99mTc-MAA. The contour of the breast is outlined by a thread-flexible 57Co source. A hot focal spot is present in the upper quadrants. The cross indicates the position of the nipple

In the event of contamination, the acquisition must be repeated after properly cleaning the skin with a decontaminant substance; in the case of spread of the uptake to the surrounding breast parenchyma, it is necessary to repeat the location of the lesion using a different method.

19.1.4 Surgery

During surgery, the lesion is localized by using a gamma probe, wrapped in a sterile sheath (Fig. 19.4): the surgeon provides then to remove the "hot spot," and the edges of the excision are defined as the locus of points surrounding the hot spot where radioactivity falls off sharply. All of the area of the tissue with a higher radioactivity count compared to the background is removed.

Once the lesion has been removed, it is therefore necessary to verify whether radioactive tracer is still on the operating table: when noted, it is necessary to extend the resection until the complete disappearance of the counting rate.

19.1.5 Results

In 2010, Veronesi and coworkers published a work which analyzed the characteristics and prognosis in 1258 women with a primary clinically occult carcinoma operated at the European Institute of Oncology between 2000 and 2006 [14].

The results obtained, after an average follow-up of 60 months, showed a low rate of local events (1.5%), of regional events (1%), and occurrence of distant metastases (1.6%), with a high rate of 5-year overall survival (98.6%). The authors therefore concluded that the radio-guided surgery was able to identify the occult lesions, allowing to make an efficient and safe breast resection with good margins of normal tissue around the primary lesion.

In a review, published in 2011, Lovricks et al. [15] analyzed 87 studies comparing WGL and ROLL: the results showed that the ROLL technique had a lower rate of positivity of resection margins, resulting in reduction of reoperation rate (combined

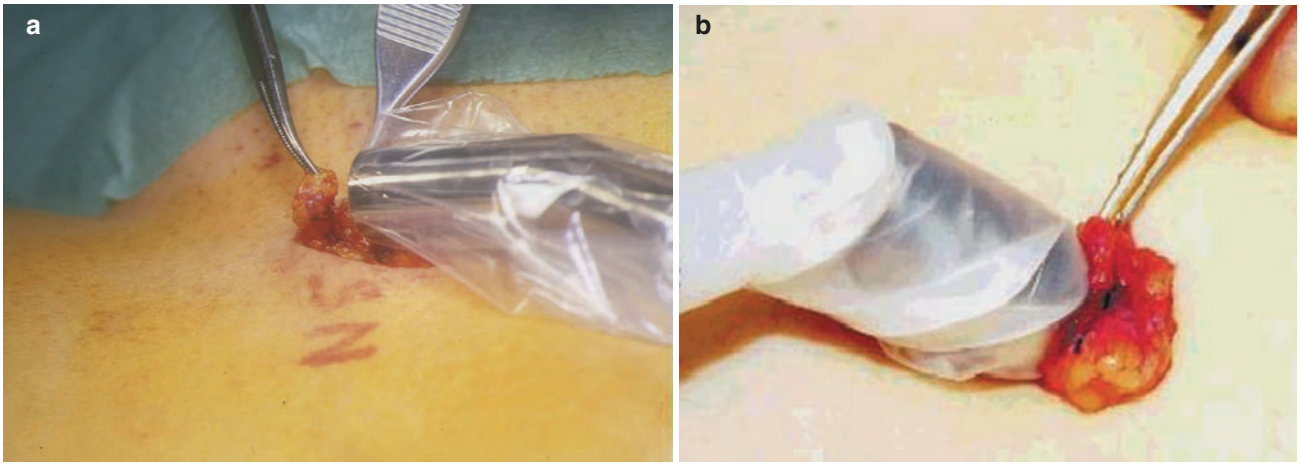

Fig. 19.4 (**a**) Lesion's localization using a gamma probe, wrapped in a sterile sheath; (**b**) lesion's counting after removal

odds ratio (OR) of 0.367 and 95% confidence interval (CI) 0277 to 0487 ($p < 0.001$) for margin status and OR 0.347, 95% CI 0.250 to 0.481 ($p < 0.001$) for reoperation rates).

Postma et al. [16], in a multicenter randomized controlled trial, compared the radio-guided occult lesion localization (ROLL) with respect to wire-guided localization (WGL), enrolling 314 patients (162 were assigned to the radio-guided localization and 152 for WGL), by analyzing primary outcomes as the percentage of complete tumor excision, the proportion of patients in need of re-excision, and the volume of the removed tissue. The authors concluded that the two procedures are comparable in terms of complete tumor excision and re-excision rates, but ROLL leads to excision of largest volumes of tissue and therefore concluded that it cannot replace WGL as the standard of care.

In evaluating the results of this study, it is necessary to consider that the radio localization method used differs from that described by the IEO group to some key features: firstly the choice of a different tracer (99mTc-nanocolloid vs. 99mTc-MAA) with a volume and a higher dose. The use of albumin nanocolloids can in fact be reflected in a larger share of lymphatic drainage and therefore in a subsequent spreading of the radioactive dose with necessity of extending the limits of the resection [17].

Recent studies have compared the ROLL with the localization method which involves the use of a titanium seed containing iodine-125 (RSL), implanted in the tumor before surgery under stereotactic or ultrasound guidance [18].

The retrospective study comparing RLS and ROLL in non-palpable breast lesions showed margin status and re-excision rates are comparable.

The RSL allows to improve the logistics for both the patient and the surgical department: in fact, in relation to long half-life of the radioactive tracer used (about 60 days), it is possible to position the seed even at a distance of time from the intervention.

Recently, Chan et al. [19] have published a review, which has considered 11 randomized controlled trials (RCTs) to assess the therapeutic outcomes of a new form of guided surgical intervention for non-palpable breast lesions against WGL, considered as the gold standard. The authors concluded that ROLL demonstrated favorable results in successful localization (RR 0.60, 95% CI 12:16 to 2:28), positive tumor margins (RR 0.74, 95% CI 12:42 to 1.29), and reoperation rates (RR 0:51, 95% CI 12:21 to 1:23) versus WGL, although the results were not statistically significant.

The authors conclude that today the WGL is still the approach most widely adopted in the location of non-palpable lesions for breast surgery. The review of the literature indicates that the ROLL can be used in clinical practice as it has proved to be a safe method, thus constituting a valid alternative to WGL with the added advantage of being able to highlight the sentinel node simultaneously.

19.2 The Sentinel Node Biopsy (SNB)

G. Paganelli

19.2.1 Introduction

The introduction of the sentinel node technique in clinical practice has resulted in a significant change in the treatment of early breast cancer, becoming the standard of care and thereby reducing the number of unnecessary axillary dissection.

The concept of "sentinel lymph node" is connected with the idea that the metastatic spread of cancer through the lymphatic follows an orderly and predictable pattern [20–22]. On the basis of this hypothesis, the histological evaluation of the "sentinel node," *which is the first lymph node that drains fluid directly from the primary tumor*, allows to exclude the presence of malignant cells in other lymph nodes. Therefore, the status of the sentinel node is able to accurately predict the pathological state of the successive lymph node stations.

During the 1990s, the concept of sentinel node showed its potential role in the surgical treatment of breast cancer [23–25]. Since then, lymphoscintigraphy has increasingly emerged as a reliable method for detecting sentinel lymph node showing success rates higher than the method so far used (blue dye). These studies started with the work published in 1993 by Alex and Krag [26] on melanoma and breast cancer to reach the optimized approach developed by our group at IEO in 1995–1996 [27] and then applied in thousands of breast cancer patients in Europe.

The first randomized trial comparing total axillary dissection versus the only sentinel lymph node biopsy was performed by Veronesi and colleagues [28], which randomized 516 patients with breast tumors smaller than 2 cm.

The study had planned to recruit 1000 for each experimental arm, but after the known preliminary results, the patients randomized to axillary dissection arm refused treatment. These results confirmed those of the NSABP B-32 study [29, 30] that showed how the sentinel node biopsy is predictive of axillary nodal status with great accuracy (96.9%), with a low false-negative rate (8.8%). In addition, the postoperative comorbidities were much less frequent in the sentinel node group.

19.2.2 Methodological Aspects of Lymphoscintigraphy

Despite its spread, there is no consensus on the methodological aspects of the sentinel lymph node procedure: there are still many controversies regarding the type of tracer to be used (different between the USA and EU), the method of injection, the type of images to perform (planar, SPECT), and the subsequent revelation in the operating room.

19.2.3 Radiotracers

The perfect radiopharmaceutical for sentinel lymph node biopsy should be easily drained from the administration area on the first node, accumulating preferentially only at this level and limiting the drainage toward the subsequent lymph node stations.

The intranodal retention is due to macrophages that line the sinusoidal spaces of the lymph nodes, whose main function is to filter the lymph-rich particulates, on the basis of an active phagocytosis [31].

After administration, the colloidal particles pass into the lymphatic circulation with a speed that is inversely proportional to particle size [32, 33].

In our experience, the ideal tracer is composed of particles with sizes between 100 and 200 nm, in order to obtain the best compromise between speed of drainage and accumulation in the sentinel node. In fact, tracer colloidal particles with sizes less than 50 nm in the lymphatic vessels drain very quickly but also pass in the lymph nodes of the second and third level (Fig. 19.5). On the other hand, the tracers consisted of too large particles (diameter >300 nm) that accumulate only in the sentinel lymph node, but with a speed of migration excessively slow.

Unfortunately the tracer colloidal particles with a diameter between 100 and 200 nm are not commercially available; currently the most widely used radiopharmaceutical in the USA is the technetium-labeled sulfur colloid in a nonfiltered (with particles ranging from about 15 to 50 nm) or filtered form, whereas in Australia and in Canada, antimony trisulfide is used (range, 3–30 nm). Many European researchers use human serum albumin particles with diameters between 40 and 100 nm (95% < 80 μm).

In our first series of 240 consecutive patients, the mean number of lymph nodes visualized using a radiocolloid particles with sizes ranging between 15 and 50 nm was equal to 2.1 (SD 1.1), while it was 1.6 (SD 0.8) for the tracer particles up to 80 nm and 1.3 (SD 0.5) with larger particles [24].

19.2.4 Injection

The optimal injection approach has been much debated in the last 20 years. The different proposed methods can be summarized into two main categories: deep injection (intratumoral, peritumoral) or superficial (intradermal, subcutaneous, or periareolar).

Several studies have been carried out to compare the results obtained with the various methods of injection. So far, only two prospective randomized clinical trials have been published [34, 35], and the results are not quite clarifiers. In fact Povoski et al. have shown that the rate of identification of the SN is greater when the injection is made intradermally, while Rodier and colleagues have shown that the most effective route of administration is the periareolar that obtains an SN detection rate of 99.11%.

Data from a study of our group [24], using both injection intradermally and peritumoral, showed no significant differences in the identification rate of sentinel node. The only difference is related to a time delay in the display of the sentinel lymph node when using the administration by peritumoral.

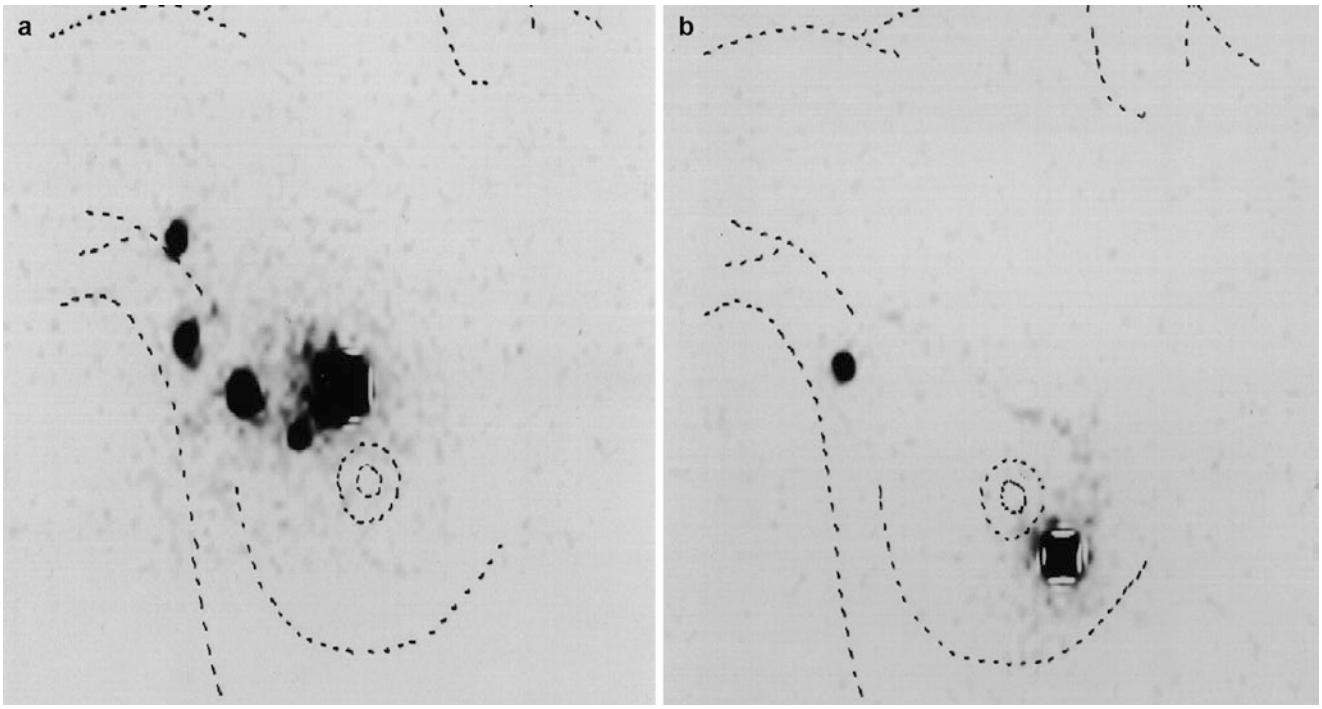

Fig. 19.5 (**a**) Scintigraphic image in the right anterior oblique projection obtained after subcutaneous injection of 99mTc-labeled sulfur colloid antimony in the upper outer quadrant of the right breast: multiple uptake areas in the ipsilateral axillary level are highlighted. (**b**) Scintigraphic image in anterior oblique projection right after subcutaneous injection of 99m Tc-labeled albumin (particle diameter comprised between 100 and 200 nm) in the inferior-inner quadrant of the right breast: it shows the presence of a single spot of uptake corresponding to the sentinel lymph node in the ipsilateral axilla

Superficial injection has the advantage of being an extremely easy method to perform, not requiring an ultrasound or stereotactic guidance for its execution also in the presence of non-palpable tumors, instead necessary in the case of peritumoral injection.

We believe that both deep and surface approach injection techniques are valid and are often complementary; the combination of both injection techniques (both peritumoral injections and retroareolar/areolar [36] or subcutaneous/peritumoral injection [37]) can improve the detection accuracy and decrease the false-negative rate (FNR). This is also supported by a study on the anatomy of the breast, lymphatic, which has shown that in some cases there are different lymphatic drainage ways, although most of the superficial lymph vessels converge in the same sentinel node [38]. Our current approach is to prefer the hypodermic injection in superficial tumors and to reserve the peritumoral administration in deep tumors.

19.2.5 Imaging

The execution of lymphoscintigraphy in the afternoon before surgery (15–18 h before surgery) is both logistically convenient for the routine in nuclear medicine and consistent with the pathophysiology of lymphatic drainage for radiocolloids with any particle size. However, when radiocolloids with small particle size are used, it may be preferable to perform the imaging 2–4 h before the surgery in order to avoid the uptake of multiple lymph node stations.

Generally, the acquisition of lymphoscintigraphy for identifying the sentinel node is performed using a large field gamma camera equipped with a high-resolution collimator. The patient should be placed in a supine position, with the arm above the head to allow placement of the gamma camera's head as close to the axilla.

The acquisition may be carried out in dynamic scan to highlight the route of drainage and then followed by the static acquisitions placing the gamma camera in anterior oblique position (+45°). Once the lymph node is displayed, it is necessary to mark the skin projection of the sentinel lymph node in the axilla using a permanent marker, positioning the arm at 90° with respect to the body in the same position of the intervention.

It seems rather unclear the added value of SPECT/CT for visualization of sentinel nodes: some authors have proposed its use especially when there is evidence of an extra-axillary drainage [39], but at present it is of little use.

19.2.6 Intraoperative Gamma Probe Counting

The intraoperative localization of sentinel nodes involves the use of a gamma probe sensor, wrapped in a sterile sheath: the sequence can vary according to the surgeon's habits, being able to perform before the removal of the primary tumor and then the sentinel node or vice versa. In patients where the primary tumor is located in the upper outer quadrant, it may be possible to use a single incision to remove the tumor that is the sentinel node.

The location of the sentinel lymph node with the gamma probe is based on the detection of a focal spot of radioactivity accumulation in the draining lymph node/s. Once the sentinel lymph node/s are localized and excised, a further search of the tracer accumulation areas must be performed to highlight the possible presence of other "hot" lymph nodes. The complete removal of the sentinel node/s is confirmed by the reduction of the rate in the axilla to background levels.

In the most recent series, the overall success rate of lymphoscintigraphy in the identification of the sentinel lymph node is very high, about 97%, higher than that of the colorimetric technique with the vital blue (mostly around 75–80%).

19.2.7 Results, Clinical Indications, and Controversies

Sentinel lymph node localization and biopsy (SLNB) represents the "standard of care" for the assessment of axillary lymph nodes in patients with breast cancer. This procedure has completely replaced the axillary lymph node dissection (ALND), in women with breast cancer in stages I and II with no clinical evidence of metastasis to the axilla [24, 40, 41].

Currently, in patients with negative sentinel node biopsy, the axillary dissection is not performed, regardless of the type of tumor present.

Axillary dissection remains, however, the standard treatment for patients with axillary metastases.

SLNB has undergone changes and improvements over the years, and the procedure is routinely performed in many situations that were considered as contraindications only a few years ago.

19.2.7.1 Minimal Lymph Node Involvement

One aspect not entirely clarified is the meaning of micrometastases and isolated tumor cells (ITC) in sentinel lymph node. Micrometastases are defined as a tumor deposit greater than 0.2 mm and/or more than 200 cells, but less than 2.0 mm, while the ITC are groups of cells not exceeding 0.2 mm or less than 200 cells [42].

Between 2001 and 2010, the International Breast Cancer Study Group (IBCSG) 23-01 recruited women from 27 institutions with breast cancer, with tumor size of ≤5 cm, and with micrometastases in the sentinel node, randomizing into two arms which included SLNB or standard treatment with ALND. After a 5-year median follow-up, the axillary recurrence rate was <1% in both arms. Survival was similar in the ALND and SLNB-alone group (DFS, 84% vs. 88%, respectively) [43].

In 2011, the St. Gallen Consensus Conference [44] recommended that the micrometastases in the sentinel node should not represent an indication for axillary dissection irrespective of the type of surgery performed.

19.2.7.2 Ductal Carcinoma In Situ (DCIS)

DCIS metastasizes to the axillary lymph nodes in a small proportion of patients (estimated at 1–2% of cases), and, if present, the meaning of these metastases is not yet clear. For these reasons, the National Cancer Institute has not recommended the use of SLNB in patients with ductal carcinoma in situ [45].

However, it was shown that approximately 40% of patients will have an underestimation of ductal invasion, so the sentinel node biopsy is currently recommended in patients undergoing mastectomy for DCIS [46]. In patients undergoing conservative surgery, the sentinel node biopsy can be performed successively in the presence of an invasion examination of the surgical samples.

However, some centers perform SLNB in patients with DCIS considering the fact that a wide local excision can cause an alteration of the lymphatic drainage, making a subsequent SLNB difficult [47].

19.2.7.3 Reoperative SLNB with Prior Breast or Axillary Surgery

The sentinel node approach has always been limited to women that have not previously undergone surgery, in relation to the fact that the lymphatic system should be intact in order to have an excellent drainage.

Published reports have shown that sentinel node biopsy can be performed after surgical treatment, either conservative or radical: in 117 patients [48], previously treated with surgery, the search for the lymph node was effective in 55% of patients, with a success rate directly proportional to the number of lymph nodes removed during the first intervention. This series revealed no locoregional recurrence after an average of 2.2 years of follow-up, but the 5% of patients developed recurrences.

Another series of 56 patients showed an 80% detection rate; after 2 years of follow-up, axillary recurrences were not reported. The same group later published a similar series in patients with a previous ipsilateral lymph node dissection, noting only a 29% success rate [49].

19.2.7.4 Pregnancy

There are several dilemmas in the management of breast cancer during pregnancy, one of which is how to assess the lymph node status of the patient. The introduction of the SLNB technique requires a careful assessment of risks and benefits in this patient population.

First, the problem that arises is linked to the use of a radioactive substance: studies have shown that the risk of radioactive teratogenicity is minimal. The SLN method both in the case of melanoma than in breast cancer usually uses doses of 0.3–3 mCi of 99mTc-nanocolloids, with a fetal absorbed dose of about 0.43 cGy [50]. It is estimated that the risk of embryonic or fetal genetic defects is equal to 0.024–0.099% per cGy [51], while the liable threshold for fetal teratogenic effects is 5 cGy [52].

Studies reported in the literature in pregnant women [50] suggest that SLNB can be performed safely in pregnancy, although data are based on currently too small populations. For this reason, the SLNB in these patients may be used, but it requires a large informed consent before embarking on this technique.

19.2.7.5 Sentinel Lymph Node of the Internal Mammary Node (IMN)

Study of the sentinel node has allowed a better evaluation of the nodal status at the level of IMN, whose valuation is generally not included in the standard surgical procedure. It was shown that the IMN metastases represent a negative prognostic factor [53], with a higher incidence of distant metastasis and reduced survival [54, 55]. The involvement of IMN is more frequent in the case of tumors located in the inner quadrants, even in the presence of subcentimeter lesions.

The risk of distant metastases increased 30% in mammary tumors located in the interior quadrants, with an increase in mortality of 20%; in particular, the risk of metastasis to the IMN is associated with the age of patients (decreases with increasing age), the size of the primary tumor, and the presence of axillary metastases [56, 57].

From a methodological point of view, the identification of IMN sentinel node requires administration of the tracer peritumorally.

This method of administration allows the display of atleast one IMN node in 60% of the tumors, while it is extremely rare that a node of the IMN is displayed using intra / subcutaneous Injection (2.1%).

However, the significance of IMN biopsy continues to be discussed. There is evidence that the mapping of IMNs brings to stage migration and treatment planning changes; however, more data are needed to support the idea that the mapping of IMNs improves treatment outcome and survival [58, 59].

19.2.7.6 ROLL and SNOLL

The sentinel lymph node biopsy (SLNB) and radio-guided occult lesion localization (ROLL) can be used in combination (SNOLL) and for cancers or high-grade-infiltrating ductal atypia.

A recent review [60] analyzed the results emerging from seven studies that evaluated 983 patients with non-palpable breast cancer. The rate of complete resection with negative margins is between 82 and 90.5%, while the need for a second operation has occurred in a percentage of between 2 and 12%.

The systematic review has shown that SNOLL is feasible, safe, and effective for the treatment of non-palpable breast cancers.

19.2.7.7 New Tracers

The techniques of SNLB and ROLL/SNOLL involve the use of radioactive substances whose use may be restricted only to the centers with a nuclear medicine department. This limiting factor is probably at the base of finding that in respect of an increased incidence of cancer, the use of the procedure of sentinel node biopsy has reached a plateau, with about 60% in developed countries who have access to this procedure [61, 62]; the percentage goes down to 5% in China and the rest of the world [63].

A recent review [64] has analyzed 21 studies investigating the use of new molecules for the research of SLN such as fluorescence of indocyanine green (ICG), contrast-enhanced ultrasound (CEUS) microbubbles, or superparamagnetic iron oxide nanoparticles (SPIO). The endpoint in this review was limited to the sentinel lymph node identification, and few data were available for other endpoints, such as false-negative and locoregional recurrence rates.

The authors conclude that there is no significant benefit of the new methods compared to the SLNB performed with radiolabeled compounds.

19.3 Positron Emission Tomography (PET)

L. Gilardi, F. Matteucci, and G. Paganelli

19.3.1 Introduction

Positron emission tomography/computed tomography (PET/CT) is an imaging modality that uses positron-emitting radiotracers associated to radiologic imaging in order to provide in vivo data on receptor and biochemical and metabolic processes of various types of tumors. The CT portion of the tomograph provides an anatomical map used for attenuation

correction of positron images and is also useful for an accurate interpretation of PET signal.

The oncological biomarker most commonly evaluated with PET/CT is fluorine 18 (^{18}F) fluorodeoxyglucose (FDG) uptake, as most malignant tumors overexpress glucose transporters and show increased hexokinase activity [65–68].

However, breast tumors have other important features, such as cell proliferation, hormonal receptor status, and HER2 status that have been explored through PET imaging with other radiotracers.

Within this chapter, we examine the actual impact of FDG-PET/CT on clinical management of breast cancer patients; moreover, we discuss future opportunities given by the development of specific non-FDG radiotracer.

19.3.2 FDG-PET/CT

19.3.2.1 Staging

An accurate staging of breast cancer patients at the time of the initial diagnosis has a major impact on the choice of the optimal therapeutic strategy [69]. Breast cancer staging includes detecting cancer spread to regional lymph nodes, both in the axilla and internal mammary chain, and also to distant sites. As a total-body procedure, PET/CT is able to assess these data all at once, providing morphological information associated to an evaluation of the metabolic activity of the disease.

There is no currently defined role for FDG-PET/CT in breast cancer detection, mainly due to its poor spatial resolution. Indeed, the sensitivity of the procedure is less than other conventional imaging modalities and depends first of all on primary breast tumor size [70, 71]. In particular, Cermik et al. found that the sensitivity of FDG-PET in detecting non-palpable, small (<10 mm), invasive malignancies ranged from 53% for T1mic and T1a tumors to 63% for T1b tumors [72].

Moreover, PET imaging accuracy is affected by tumor histology: lobular and ductal intraepithelial neoplasia (LIN and DIN) can be missed, and invasive lobular carcinomas are detected with less sensitivity than ductal carcinoma, due to their pattern of growth [73, 74].

Actually, the main contribution of PET in primary breast tumor assessment consists in measuring FDG uptake through the standardized uptake value (SUV), which is useful to evaluate the subsequent response to neoadjuvant therapy in cases not submitted directly to surgery, even at an early point during treatments. Moreover several studies reported the correlation between SUV of breast cancer and prognostic parameters such as tumor size, axillary lymph node involvement, negativity of estrogen receptor expression, high tumor grade, and HER2 overexpression [75–77]. An association has also been found between primary tumor FDG uptake and immunohistochemical-defined subtypes of breast cancer, more biologically aggressive tumors, i.e., HER2 positive and triple negative, demonstrating higher SUV values than luminal ones [78, 79].

An evolution of PET in this setting is the development of positron emission mammography (PEM) that is a relatively new technique being investigated for use in breast cancer diagnosis. After FDG injection, the breast is positioned in a device similar to a mammography cassette. PEM demonstrated improved sensitivity if compared to whole-body PET/CT, in particular for detection of small lesions [80]. Other benefits are that PEM is relatively unaffected by breast density and that it is able to detect in situ lesions [81], suggesting a potential role in high-risk patients, in women with dense breast, and in the diagnosis and postsurgical follow-up of intraductal lesions.

For staging of the axilla, it has been demonstrated that PET/CT cannot be used as a substitute of sentinel node biopsy (SNB) due to the limited spatial resolution that precludes an adequate evaluation of small size lymph nodes, of metastatic lymph nodes with few FDG-avid cells, and of the axillas with few involved nodes. Sensitivity values as low as 20% have been found in some series [82–84]; in particular, in a study by Veronesi et al., only 37% of 236 patients with clinically negative axilla and with a positive sentinel node biopsy had FDG-positive axillary lymph nodes at presurgical PET scan [82].

On the other hand, a node-positive PET scan has high specificity and positive predictive value for axillary staging and indicates a higher disease spread to this region [85, 86]. Therefore PET/CT is useful in differentiating low- vs. high-burden nodal disease and could guide the choice of surgical treatments on the axilla: direct axillary lymph node dissection (ALND), foregoing SNB, has been proposed in PET node-positive patients, while SNB actually remains the choice as a staging procedure in PET node-negative cases.

In contrast with the limited accuracy in axillary lymph node staging, as a total-body procedure, PET/CT has a great value in the diagnosis of extra-axillary lymph node metastases and distant sites of disease (Fig. 19.6). The detection of Berg level III (infraclavicular) or extra-axillary local-regional (supraclavicular or internal mammary) nodal disease or of distant metastases has important implication in surgical and radiation therapy planning and in the definition of the real aim (curative vs. palliative) of therapeutic strategy in newly diagnosed breast cancer. Some studies have demonstrated that PET is superior to conventional imaging in this setting [87–90]. Ng et al. detected occult metastases in 17/154 patients (11%); locoregional nodal spread missed by conventional imaging was instead found in 15/154

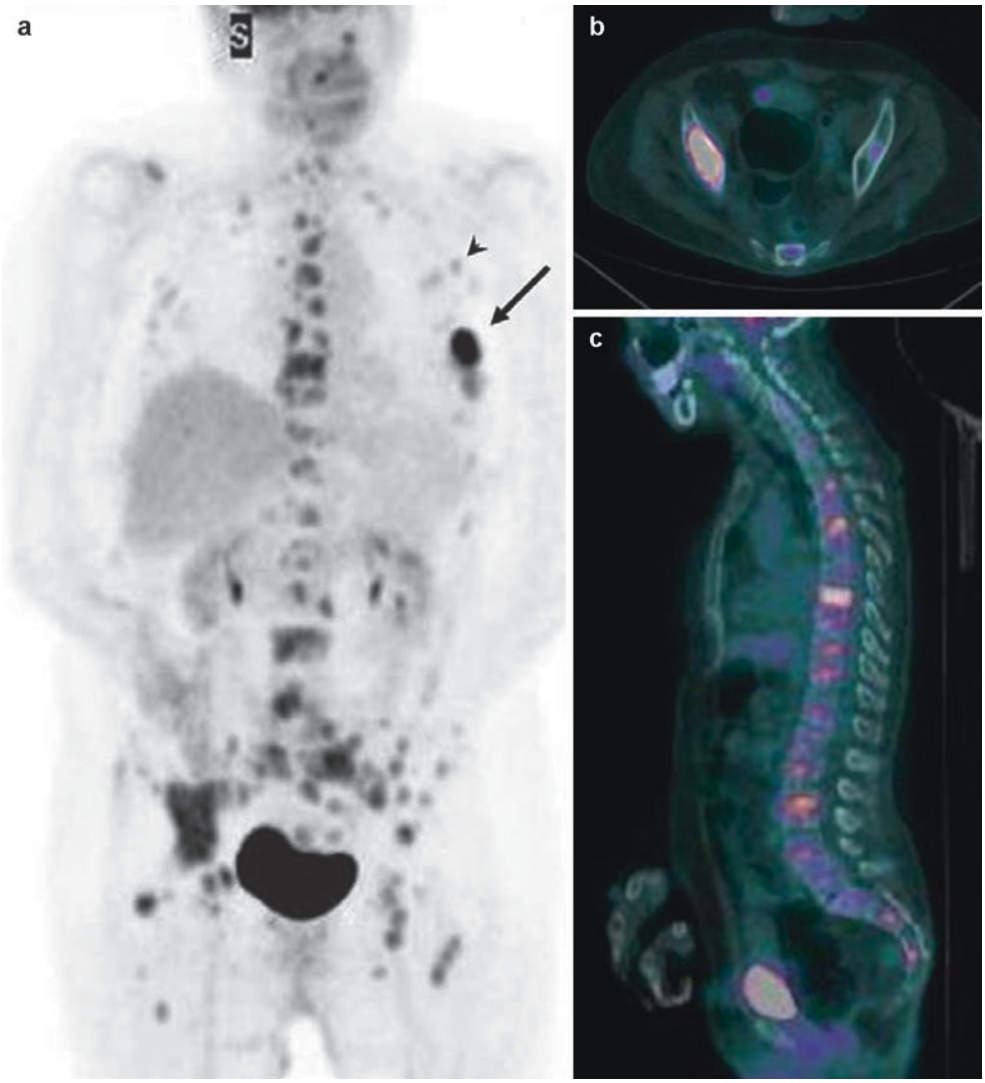

Fig. 19.6 Staging PET/CT scan in a 62-year-old woman with invasive ductal carcinoma of the left breast (cT2N1, ER 90%, PgR 90%, HER2 negative, Ki67 28%). (**a**) Maximum intensity projection image shows FDG uptake in primary breast tumor (arrow) and axillary lymph nodes (*arrowhead*), associated to multiple, hypermetabolic lesions in the bone. (**b**) Axial PET/CT fusion image shows FDG uptake in pelvic bones. (**c**) Sagittal PET/CT fusion image shows multiple foci of uptake in the spine

patients (10.8–13% had ipsilateral internal mammary node involvement and 2% ipsilateral supraclavicular nodal metastases), leading to a change of the radiation treatment field [88]. Likewise, in 13/154 (8%) and 7/70 (10%) patients, there was a therapeutic change due to PET/CT detection of occult metastases in Koolen et al. and Segaert et al.'s studies, respectively [89, 90].

PET/CT is a valuable tool in locally advanced breast cancer, but also in early-stage disease [90–93]. In a study by Groheux et al., PET/CT demonstrated a nonnegligible yield in patients with stage IIB and primary operable stage IIIA breast cancer. In these patients with T3N0, T2N1, or T3N1 disease, the overall yield was 13% with a change in therapeutic management due to the finding of N3 disease or distant metastases. Moreover, 2 of 36 patients with stage IIA disease were upstaged due to the discovery of internal mammary lymph node (one patient) and contralateral supraclavicular and mediastinal nodal disease (one patient) [92]. Extra-axillary lymph node involvement was also detected in almost one third of stage II–III breast cancer patients in the study of Aukema et al. PET/CT upgraded the TNM stage in 10/60 patients (17%), with a change of the radiotherapy plan in 7/60 (12%) [94].

These studies demonstrate that FDG PET/CT imaging is applicable to a patient population with a wide range of breast cancer stages, including T1–T4 carcinomas. Nevertheless, actually some uncertainties remain about the

exact characteristics (clinical TNM stage and histopathological features) of newly diagnosed breast cancer for which this imaging procedure should be systematically performed with a favorable cost-effectiveness balance. Targeted prospective studies with a large number of patients are necessary to better define this point.

19.3.2.2 Monitoring Response to Therapy

It is well known that changes in metabolic activity generally occur earlier during treatments than those in tumor size. This is particularly true for new targeted therapies that are more cytostatic than cytotoxic and that can render tumors metabolically inactive without significant changes of their morphological aspect. Moreover, in the specific case of breast cancer, common sites of dissemination such as bone metastases, pleural effusion, and lymphangitis are difficult to assess with conventional imaging.

Even if the specific criteria for therapy response assessment proposed by various working groups are not actually accepted and widely used [95, 96], PET/CT demonstrated to be a valuable tool in this setting.

In particular, in the case of bone metastatic breast cancer, FDG-PET/CT is emerging as a standard of care.

Initial reports found that FDG-PET and bone scintigraphy had similar sensitivity for the detection of bone metastases, ranging from 57 to 100%, while the specificity was superior for PET, approaching 96–100%. These data reflect inherent limitations of the two imaging procedures, as bone scintigraphy poorly detects osteolytic lesions and PET is often less sensitive in pure osteoblastic metastases [97–99]. However, the development of integrated FDG-PET/CT has improved the accuracy in the detection and response evaluation of bone metastases by adding information on bone morphological changes.

As regards morphologic imaging, it does not directly reflect tumor cell viability but rather the secondary effect on bone adjacent tissue. Afterward, morphologic changes are often delayed during therapy [100] and do not seem to correlate with the presence of residual active tumor [101]. Change in tumor size is also not a good surrogate of bone lesion response, and the RECIST 1.1 criteria specify that bone lesions without soft tissue components cannot be considered as measurable [102]. Moreover, a "flare" reaction can be assessed on CT or bone scan, expression of the sclerotic healing, making the response evaluation difficult or even leading to a misinterpretation of the disease course [103, 104].

PET/CT is not burdened by these limitations, as FDG uptake reflects the metabolic feature of bone metastases independently of their CT pattern (osteoblastic and osteoclastic) [101] (Fig. 19.7).

As a total-body procedure, PET/CT is also able to provide information about all sites of disease in a single test; response to therapy may indeed be heterogeneous, with the coexistence of responding and not responding lesions within the same patient. Huyge et al. studied metabolic response by comparing PET/CT scans carried out before and during a new treatment phase in 25 bone-dominant metastatic breast cancer patients and found that, in the subset with both bone and extra-bone metastases, PET/CT showed discordant responses between bone and extra-bone metastases in 30% of treatment phases. Moreover, a heterogeneous metabolic response seemed to have prognostic implication, as time to progression was longer in patients with a heterogeneous nonresponse of bony lesions compared with those with a homogeneous nonresponse [105].

In the neoadjuvant setting, several studies have shown promising results for the early prediction of pathological complete response of breast cancer using PET/CT, even after only one cycle of therapy [106–108]. The early prediction of response during neoadjuvant therapy might offer the opportunity to change strategy in case of ineffectiveness, avoiding unwarranted side effects at the same time.

An impact of the different breast cancer subtypes on FDG uptake during neoadjuvant treatment has been reported, with conflicting results. For example, in Koolen et al.'s study, PET/CT resulted predictive of response in ER-positive/HER2-negative and triple-negative tumors but was less accurate in HER2-positive tumors [109], while Humbert et al. found that the metabolic imaging procedure was efficient in the determination of final pathological complete response only in patients with HER2-positive tumors [108]. Moreover, Groheux et al. found that quantitative indexes derived from interim FDG-PET/CT that are best correlated with pathologic response vary by subtypes, opening new areas of investigation.

Despite the growing evidence supporting the use of PET/CT in this field and the benefits that would result for patients, its use has not yet entered into the routine. This is mainly due to the heterogeneity of the available studies, in particular with regard to the timing of the interim evaluation and to the degree of SUV decrease used to define the response.

19.3.2.3 Recurrent Breast Cancer

Early diagnosis and correct localization of recurrent breast cancer are important to allow the appropriate treatment that

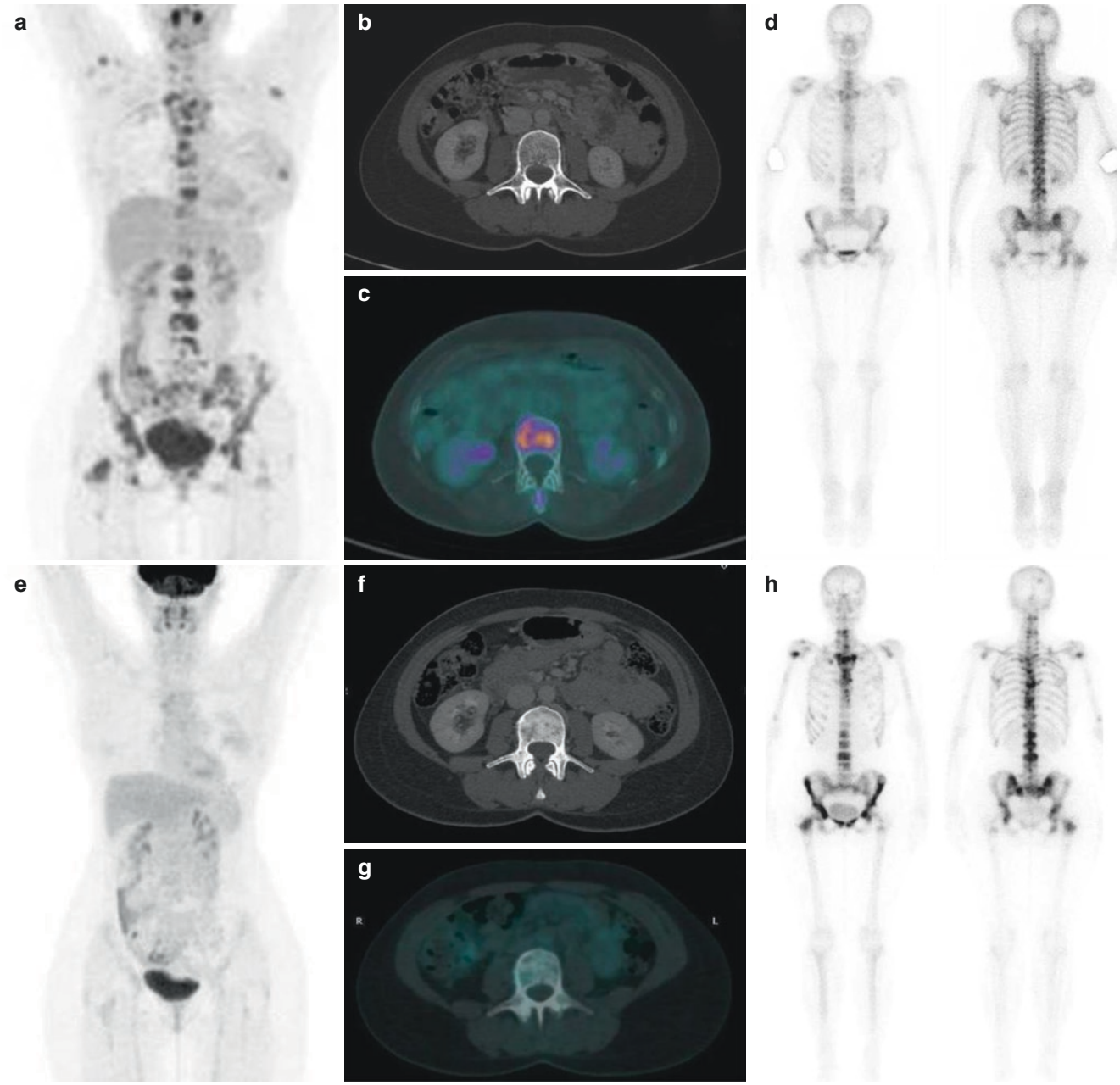

Fig. 19.7 A 43-year-old woman treated with surgery, chemotherapy, and radiotherapy due to left breast cancer in 2013. Follow-up PET/CT demonstrated diffuse bone disease (maximum intensity projection image and fused PET/CT axial image that refers to L3 (**a**) and (**c**), respectively) that was confirmed, with lesser extent, by a bone scintigraphy (**d**). Contrast-enhanced CT detected only few of the bone lesions; in particular L3 didn't show significant alterations (**b**). After chemotherapy and administration of zoledronic acid, PET/CT was negative (maximum intensity projection image and fused PET/CT axial image that refers to L3 (**e**) and (**g**), respectively). On the contrary, bone scintigraphy (**h**) and CT scan (axial image that refers to L3 (**f**)) showed an increase of foci of abnormal uptake and of osteoblastic bone lesions, respectively ("flare" reaction)

could offer the better prognosis and a good outcome. Disease recurrence may be suspected owing to the presence of symptoms or increase of tumoral markers or because of radiologic findings. PET/CT has been shown to perform better than conventional imaging in all these different settings [110–113]. This is not unexpected, as FDG-PET is a molecular imaging technique that detects metabolic changes in tissues. Since functional changes precede morphological changes,

FDG-PET has the potential to detect viable tumor tissue earlier in the course of disease evolution.

In particular, PET/CT showed an important role in asymptomatic patients with rising tumor marker levels and negative conventional imaging results. Grassetto et al. studied 89 breast cancer patients that met these characteristics. Tumor deposits were detected in 40/89 patients in the chest wall, internal mammary nodes, lungs, liver, and bone; in 23/40 patients, solitary small lesion was amenable to radical therapy [110].

Chang et al. performed PET/CT on 71 patients with increased serum CA15-3 levels and/or clinical/radiological suspicion of recurrence and, as control group, on 69 asymptomatic breast cancer patients in their post-therapy surveillance. Recurrences were proven in 56.3% (40/71) of the patients with suspected recurrence, but also in 13% (9/69) of the control group [114].

Moreover, PET/CT has the major advantage of evaluating the entire body in a single procedure and the possibility of determining whether the recurrence is isolated or not, providing essential information for patients' management. In Aukema et al.'s study, 56 patients with confirmed locoregional recurrence were evaluated to visualize the extent of recurrence and to exclude distant involvement. PET/CT confirmed all the known sites of disease and depicted additional lesions not visible on conventional imaging in 25 patients (45%). The procedure had an impact on clinical management in 27 patients (48%) by detecting more extensive locoregional disease or distant metastases. In 20 patients (36%), extensive surgery was prevented, and treatment was changed to palliative therapy [115]. Similarly, even in other studies, PET/CT results have been shown to have a great impact on management of patients with suspected recurrence, leading to changes of the treatment modality or intent in 48–54% of the cases [112–114].

Therefore, PET/CT appears to be the imaging modality of choice in this category of patients and should be performed as early as possible in case of any suspicion of recurrence or metastases.

19.3.3 PET/CT with Non-FDG Tracers

As previously reported, ^{18}F-FDG is not the only radiopharmaceutical available to evaluate patients with breast cancer. Other important features of this tumor have already been tested in human with novel PET tracers such as ^{18}F-fluorothymidine (^{18}F-FLT), ^{18}F-fluoroestradiol, and anti-HER2 radiopharmaceuticals that may provide additional useful information about breast cancer marker expression, heterogeneity of the disease, and responsiveness to therapy [116–119].

FLT has been developed to evaluate an increased cellular DNA synthesis and a good correlation between the tracer uptake and Ki-67 labeling index of breast cancer that has been reported [120]. FLT-PET may not be useful for staging purposes, as the tracer has a high physiological uptake in the liver and bone marrow. However, encouraging results have been reported in the early evaluation of response to therapy, even a week after initiation of chemotherapy [121, 122].

^{18}F-fluoroestradiol, that binds to estrogen receptors (ER), has been so far the most studied of the new tracers: its uptake demonstrated a correlation with ER expression in primary breast cancer tumors but even in locoregional lymph nodes and distant lesions (Fig. 19.8) [111, 123].

FES-PET could therefore be useful in the assessment of ER expression heterogeneity (as metastases can display different characteristics that could also not match those of the primary tumor [124]), leading to the evaluation of the entire tumor volume receptor status through a single, non-invasive procedure, bypassing the possibility of an error in the pathological determination of ER and assisting the individualized treatment decisions. Sun et al. and Van Krutchen et al. investigated the value of FES-PET in breast cancer patients presenting with a clinical dilemma. Both studies included 33 patients and found changes in treatment plans due to the PET results in 48% of the cases [125, 126].

FES uptake in disease sites has also been shown to be predictive of response to therapy. Linden et al. studied 47 heavily pretreated metastatic breast cancer patients with immunohistochemical ER-positive tumors and found that none of the 15 patients with initial SUV less than 1.5 responded to hormonal therapy, compared with 11 of 32 patients (34%) with SUV higher than 1.5 ($p < 0.01$) [125]. FES-PET could be used to identify patients unlikely to obtain an objective response and could lead to the exclusion of an ineffective treatment.

Finally, preclinical results with tracers with high specificity for HER2, such as ^{68}Ga-labeled affibody and ^{89}Zr-trastuzumab, are abundant and promising, but clinical data are still limited to small series of patients [112, 113, 126]. Further study is needed to identify the best radiopharmaceutical in this setting (concerning the optimal dosage, the isotope that should be used, and the time of image acquisition) and to define the real impact of HER2-PET on clinical management of breast cancer patients.

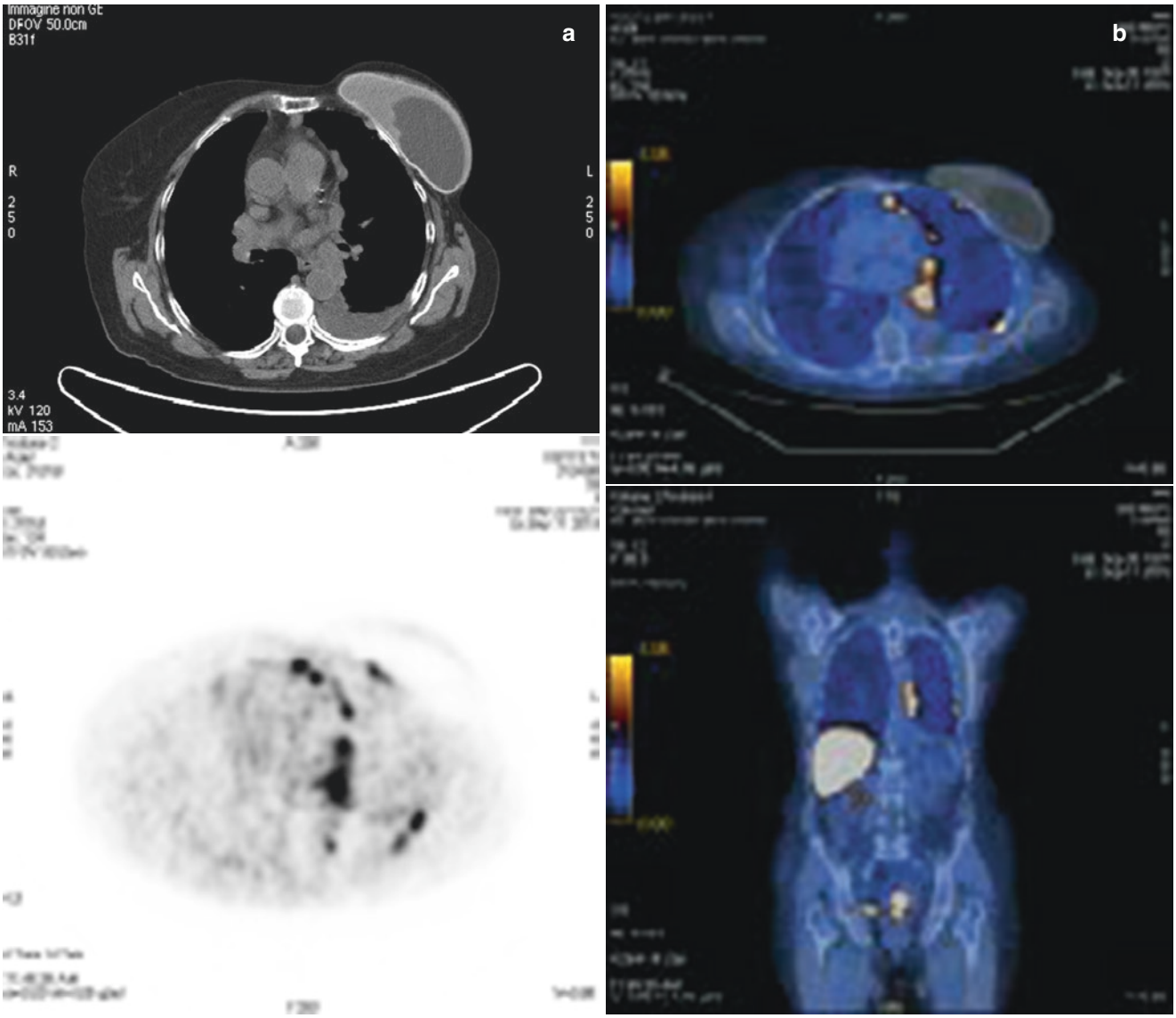

Fig. 19.8 A 79-year-old woman treated with surgery, chemotherapy, and radiotherapy due to left breast cancer in 2008 (invasive ductal carcinoma of the right breast; G3, pT1cN1M0 ER 100, PR 15%, Mib1 15%, HER2 not amplified).

Conclusion

In conclusion, FDG-PET has a big impact at different phases of the disease in breast cancer patients, from staging to assessing response to therapy or suspected recurrence. At present, many efforts have to be made to standardize study methodology, in order to allow the results to be compared and reported widely in clinical practice. It will also be important to consider the disease heterogeneity, with the aim of target studies on subpopulations that show inherent differences in prognosis and treatment (subtype-tailored PET imaging). Finally, the implementation of new PET tracers, with an overall in vivo assessment of the different characteristics of the disease, could help in the definition of the optimal therapeutic strategy. A combined, personalized determination of glycolytic activity and molecular marker expression may therefore become the basis for treatment of individual patients with different subtypes of breast cancer.

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One-Step Systemic Staging for Patients with Breast Cancer

20

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20.1 Background

Despite advances in the treatment of primary breast cancer, metastatic spread of the disease remains a substantial clinical burden. Nearly 30% of breast cancer patients already have tumour spread to regional lymph nodes at diagnosis, and 5% will have metastases at presentation [1]. The prevalence of metastatic disease has increased along with the duration of survival, with some 20% of patients developing metastases during the course of the disease [2].

Breast cancer commonly metastasizes to the lymph nodes, bone, liver, lung and the central nervous system [3]. Of these, bone is the most frequent, being the first site of metastasis in more than 50% of the cases of relapsing disease [4], and present at the time of death in over 70% of those patients who die of breast cancer [5].

In order to effectively manage metastatic breast cancer patients, it is essential to have consistent, reproducible and validated methods for the detection of metastatic disease and for the evaluation of therapy response. These methods include clinical assessments, serum biomarkers and imaging techniques.

20.2 Clinical Assessments

Clinical assessments addressing pain, energy levels and mobility, often by means of questionnaire tools [6], are for the most part in the form of structured measures of quality of life. Although widely used in clinical trials, these questionnaires are not integrated into daily practice.

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20.3 Serum Biomarkers

The serum biomarkers applied to the evaluation of metastatic breast cancer include CA 15.3, the oncofoetal protein carcinoembryonic antigen (CEA), the oncoprotein HER-2/neu and the cytokeratin tissue polypeptide-specific antigen (TPS) [7]. Although serum biomarkers are helpful in the detection of recurrent disease, they perform variably in the evaluation of treatment response [8] and are not useful for evaluating heterogeneous response across different metastatic sites.

Serum biomarkers of bone heath are complementary and seem to be particularly useful in patients who have bone disease that is difficult to assess by means of other methods [9]. Markers such as N-telopeptide of type I collagen (NTX) and bone-specific alkaline phosphatase (BAP) are related to osteoclastic/osteoblastic bone activity, respectively, and their elevation or reduction (in the case of NTX) has been related to increased or diminished risk of developing skeletal-related events, as well as being correlated to survival [10, 11].

Of increasing recent interest for assessing therapy response is the use of circulating tumour cells (CTCs) and circulating tumour cell-free DNA. It has been demonstrated that levels of CTCs at baseline and after chemotherapy are predictive of progression-free survival and overall survival in metastatic breast cancer [12, 13]. However, changing therapy on the basis of persistently elevated CTC levels despite treatment does not bring an increase in overall survival [14]. CTC evaluations have demonstrated an advantage over conventional radiological studies for predicting overall survival, but show low correlation with radiographic tumour load [13]. Circulating tumour cell-free DNA has shown early promise, and there is evidence of superiority to CTCs, in small studies of treatment response assessment in metastatic breast cancer [15]. Guidelines from the International Consensus Conference for Advanced Breast Cancer, therefore, state that changes in serum biomarkers alone should not be used alone to initiate changes in treatment [16].

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20.4 Imaging

20.4.1 Bone Scintigraphy (BS)

Planar bone scans with 99mTc-MDP (technetium-99m-methylene diphosphonate) are useful for the identification of metastatic bony disease as they have acceptable sensitivity [17]. Modern extensions to BS with SPECT or CT-SPECT improve bone scan performance [18, 19]. It is important to remember that 99mTc-MDP is bound to the bone as part of osteoblastic activity [20], and so BS does not necessarily reflect the full burden of disease within bone marrow space. In particular, pure lytic bone changes without an osteoblastic reaction may be missed (Fig. 20.1). In addition, it is impossible to assess patients with very advanced bone disease objectively because new disease cannot be confidently identified on the background of already elevated bone scan uptake (so-called superscans).

The utility of BS to positively identify response (as opposed to stable/progressive disease) is severely limited, because reductions of bone activity occur late in responding patients, which compromises the timeliness of bone scan readouts. Moreover, it is recognized that isotope BS can show transient increases in the size of detected lesions or new lesions in patients who are later shown to be responding to therapy (*flare reaction*) [21]. The biological explanation for the flare reaction is that successful treatment leads to osteoblast healing, which increases MDP uptake. The evaluation of response to therapy using BS is thus indirect (not reporting on tumour cell kill), and there is no evidence that bone scans may be used to assess positive therapy benefits [22].

20.4.2 Computed Tomography (CT)

Computed tomography (CT) is superior to bone scintigraphy for detecting bone disease [18]. CT scans allow measurement of the size of body metastases, extent of disease involvement and quantification of response to treatment, particularly of soft tissue disease. While CT measurements of soft tissue disease are incorporated into the RECIST [23], bone metastases are considered non-evaluable/measurable according to these criteria. Under RECIST, therefore, CT scans are used to assess response to treatment only for those bone metastases that have a measurable soft tissue component. The MD

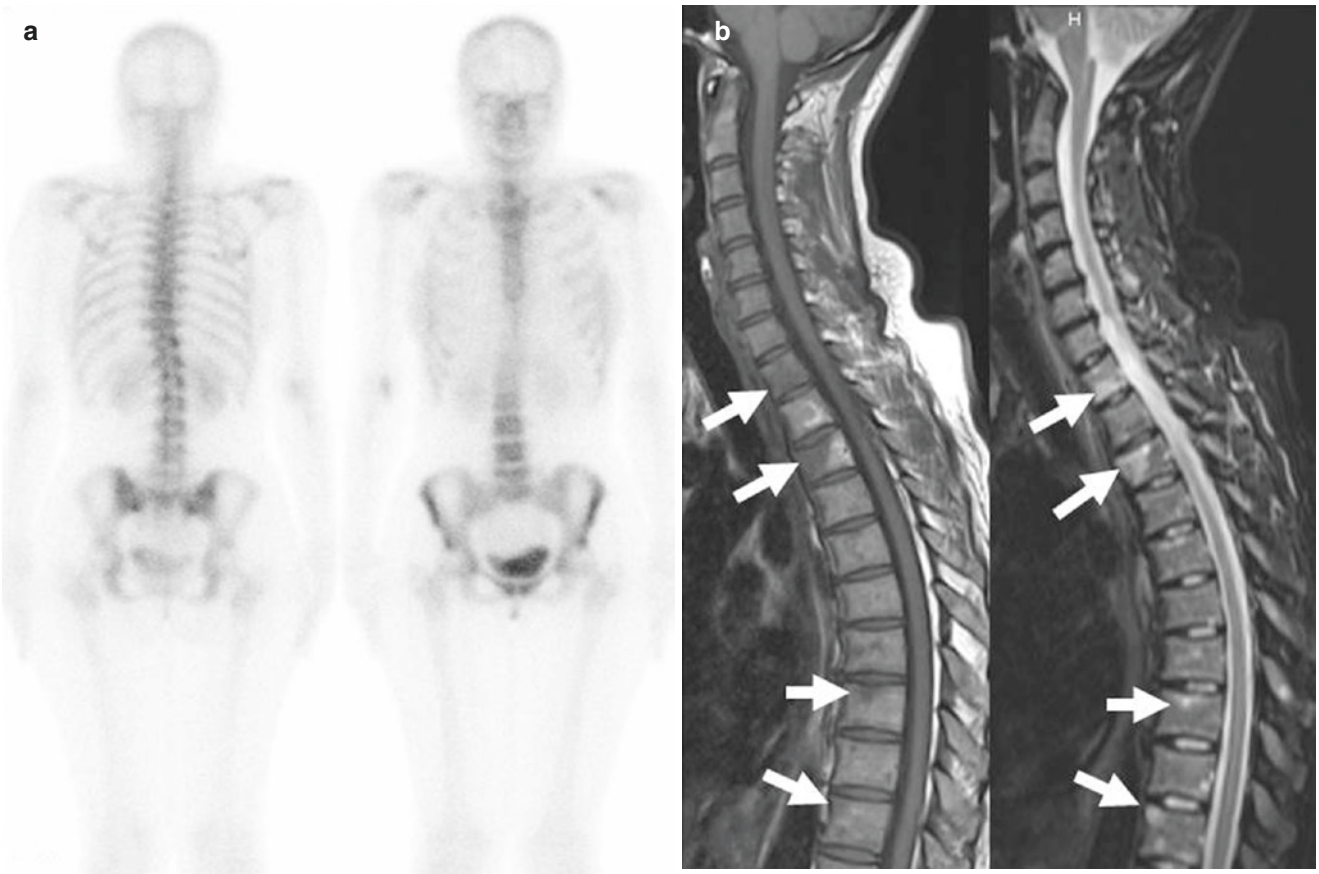

Fig. 20.1 Pure lytic bone metastases may not be apparent on bone scans. In this 43-year-old woman with nodal and liver metastases from breast cancer, 99mTc-MDP results were negative (**a**). In T1- and T2-weighted MRI of the spine performed 5 days later, multiple bone deposits can be seen (**b**—*arrows*). The large metastases in the thoracic spine show distinctive lytic features on MRI (*arrows*)

Anderson Cancer Centre criteria [24] have defined other CT features for defining response to treatment, which are based on the changes in bone structure and density within lesions. According to these criteria, an osteosclerotic reaction of a lytic/infiltrative lesion can be used as an indicator of response, as it can represent a healing process which again needs osteoblastic action (Fig. 20.2). Using the MDA criteria, the development of new osteosclerotic lesion(s) should not be classified as progression unless there is other evidence of disease progression. Unfortunately, these criteria have a significant limitation, as they are not applicable in breast cancer patients who receive anti-osteoclastic therapy (bisphosphonates), which are standard of care medicating in the metastatic setting.

20.4.3 Positron Emission Tomography (PET)

Positron emission tomography (PET) is an established technique for the diagnosis of distant metastases in breast cancer. It has potential advantages over anatomical imaging in that it demonstrates changes in metabolic activity that may occur prior to the changes in morphology depicted in CT. PET offers several different radiotracers for bone (^{18}F-NaF) and bone marrow imaging (^{18}F-FDG is the most commonly used marrow agent in breast cancer). ^{18}F-FDG PET has a strong role in evaluating metastatic disease that has accelerated glucose metabolism [25]. Unfortunately, in up to 42% of all oncological patients have FDG non-avid disease that is not appropriate for evaluation with ^{18}F-FDG PET [26]. In the setting of breast cancer, lobular cancer is oftentimes ^{18}F-FDG PET negative. ^{18}F-FDG PET data acquisition is usually coupled with CT for attenuation correction and anatomical correlation. The overall sensitivity and specificity for skeletal metastases detection of ^{18}F-FDG PET/CT are superior to those of CT and BS [27]. The role of PET/CT for monitoring bone response to therapy has been reported in a few, promising but small-scale studies [28]. Amongst the recognized limitations include the *flare* phenomenon; bone marrow "flare" reactions have been described for FDG PET/CT when bone marrow growth factors such as granulocyte colony-stimulating factor (G-CSF) are administered. In specific cases, the observation of a flare reaction could indicate therapy success, such as after the start of tamoxifen/fulvestrant therapy (usually after 7–10 days) in oestrogen receptor-positive breast cancers [29, 30].

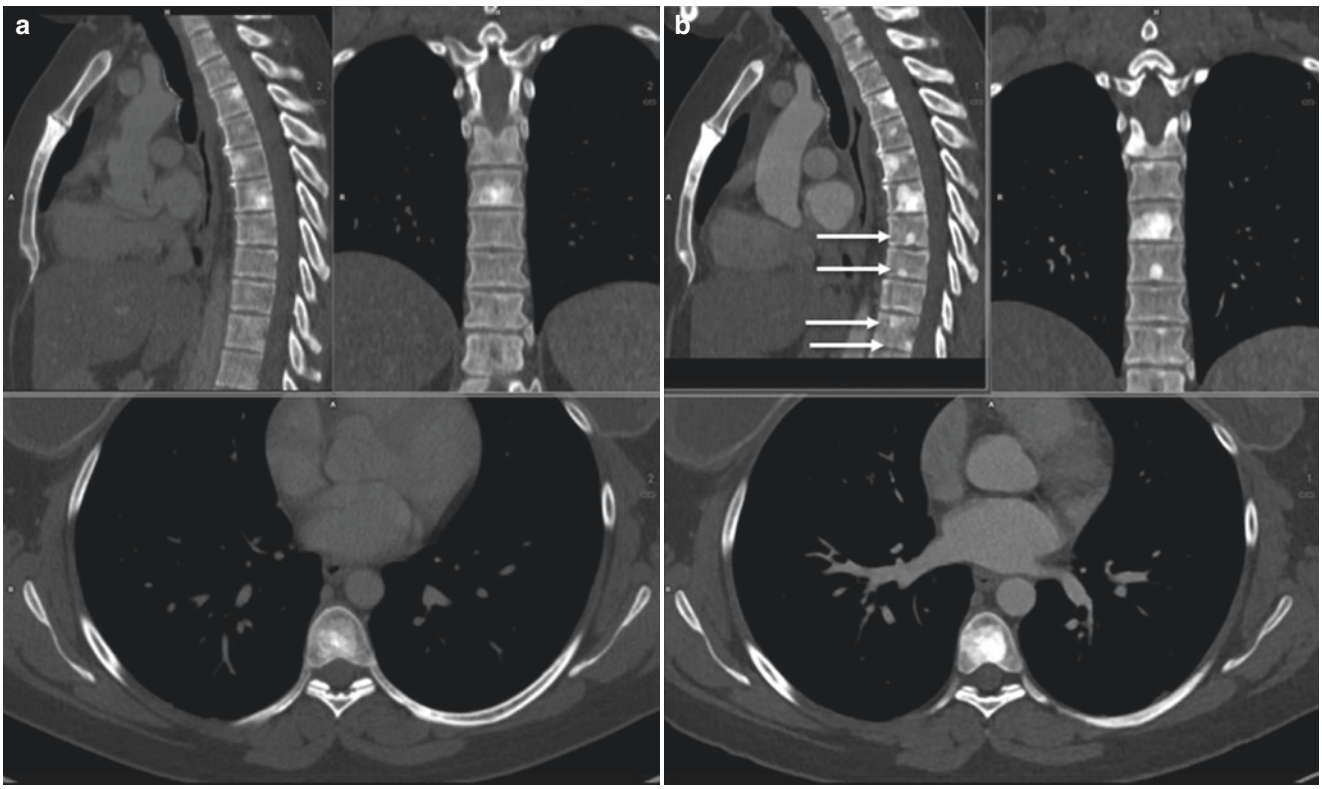

Fig. 20.2 Sclerotic response of bone metastases to therapy. A 35-year-old woman with BRCA-positive breast cancer and skeletal metastases has received prior, ineffective therapy with poly-ADP ribose polymerase (PARP) inhibitors. CT scans are acquired before and after new treatment with three cycles of carboplatin and bisphosphonates. Multiple metastases are visible in thoracic spine, some of them showing mixed sclerotic/lytic features (**a**). Dense sclerotic reaction can be seen in (**b**) in all lesions, likely indicating effective response to therapy. New, small sclerotic lesions have appeared in the second scan (*arrows*), suggesting response to therapy of smaller metastases that are not present/visible in (**a**)

20.4.4 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) has a growing role in the diagnosis and assessment of response of metastatic disease and in particular bony disease. The key advantage of MRI is that the bone marrow can be directly evaluated using a variety of sequences each sensitive to different aspects of bone and bone marrow, such as the marrow cellular density (diffusion-weighted imaging (DWI) MRI), vascularity (dynamic contrast-enhanced (DCE) MRI) (diffusion-weighted imaging (DWI)), trabecular bone density (ultrashort echo time MRI and susceptibility-weighted MRI) and bone marrow fat:water ratio (Dixon MRI, MR spectroscopy). Another advantage of MRI is the ability to perform multiregion examinations including whole-body studies. Furthermore, techniques can be combined thus enabling morphologic and functional (sometimes quantitative) assessments of tumour response, which can be repeated as required, as there is no radiation exposure penalty. Advantages of MRI include the fact that no ionizing radiation is administered, no injection of isotopes is necessary and whole-body examinations are possible.

Several meta-analyses show that the performance of MRI is comparable to ^{18}F-FDG PET, both being significantly more accurate than bone scintigraphy and CT for detecting bone metastases in many types of cancers, on a per patient and per lesion basis [27, 31, 32]. MRI also performs well for monitoring therapy response of metastatic breast cancer patients using bone-specific response criteria [33]. Progression criteria include increase in number/size of focal/diffuse areas of metastatic infiltration within normal marrow, evolution of focal lesions to a diffuse neoplastic pattern and the appearance of or increases in soft tissue components associated with bone disease. The appearance of new fractures (needing radiotherapy/surgical interventions) should be considered as progression only if the bone marrow MRI signal intensity in the affected area is indicative of malignancy.

Amongst the findings considered indicative of bone lesion response are the emergence of intra-/peritumoural fat within/around lesions (*fat dot* and *fat halo* signs), decreases in contrast enhancement and the development of dense lesion sclerosis on T2-weighted fat-suppressed MR images.

There is however limited evidence for the use of morphologic MRI criteria for the assessment of bone response to treatment. Instead, a few small studies have identified problems with the use of morphologic descriptors of response, including arrested resolution of abnormalities despite effective therapy (presumed to be due to bone sclerosis, due to marrow fibrosis or due to necrosis). Other limitations of morphologic imaging include the problem of evaluating disease activity against an already scarred background and the so-called "T1W image pseudoprogression" phenomenon that occurs due to intense bone oedema secondary to massive cell death and inflammation, which can lead to darkening of the bone marrow on T1-weighted sequences, mimicking metastatic spread through the affected segments. Ollivier and colleagues have described these technical bone marrow changes in some detail [34], but the clinical data for the use of morphological MRI in the routine assessment of metastatic bony disease response are still lacking.

20.4.4.1 Diffusion Whole-Body (DWB) MRI

Diffusion whole-body (DWB) MRI is emerging as a promising bone marrow assessment tool for detection and therapy monitoring of bone metastases [35–37]. DWB MRI continues to make use of anatomic T1 and T2 sequences for morphologic evaluation, but combines them with diffusion-weighted sequences, for the functional representation of cellular density within tissues (Fig. 20.3). Diffusion-weighted imaging evaluates the microscopic motions of tissue water and allows the calculation of water diffusivity (apparent diffusion coefficient (ADC)) that reflects the degree of freedom of water movement. Water diffusivity is determined by architectural tissue properties such as cellular density, cellular arrangements, vascularity, size of the extracellular space, tissue viscosity and nuclear:cytoplasmic ratio. Increased tumour cell proliferation tends to increase cell density while decreasing the volume of the extracellular space, resulting in reductions of ADC values [38]. Importantly, due to technological advances, DWB MRI can be performed in clinically acceptable examination times (20–30 min depending on scanner capabilities); actual scan times are longer when combined with morphologic sequences (generally 40–50 min) [35–37].

20.4.4.2 Detection of Metastases

DWB MRI is attractive for metastatic lesion detection because diffusion-weighted imaging permits at a glance assessments of the entire body, immediately drawing attention to potential abnormal skeletal and body regions and thus helping to reduce image interpretation times of anatomic MRI [36]. On diffusion-weighted imaging, lytic/infiltrative skeletal metastases appear as focal or diffuse areas of high-signal intensity on high b-values (such as b900 s/mm^2, i.e. strongly weighted diffusion images) on a background of lower signal intensity of the normal bone marrow. It is important to emphasize that metastasis detection on diffusion-weighted images should not be done in isolation but rather has to be considered as a potent adjunct to the anatomical MRI assessments, the combination of which form a complete DWB MRI assessment [39]. This assertion has been highlighted in a recent meta-analysis demonstrating that DWI alone is a sensitive but rather unspecific tool for the detection of bone metastases [40]. Thus, the pooled sensitivity/specificity of DWI alone has been reported as 87.7% (95% confidence interval [CI]: 76.3–94.9%) and 86.1% (95% CI: 79.2–91.4%), compared to 90.9% (95% CI: 84.3–95.4%) and 96.1% (95% CI: 92.2–98.4%) for whole-body MRI without DWI [40].

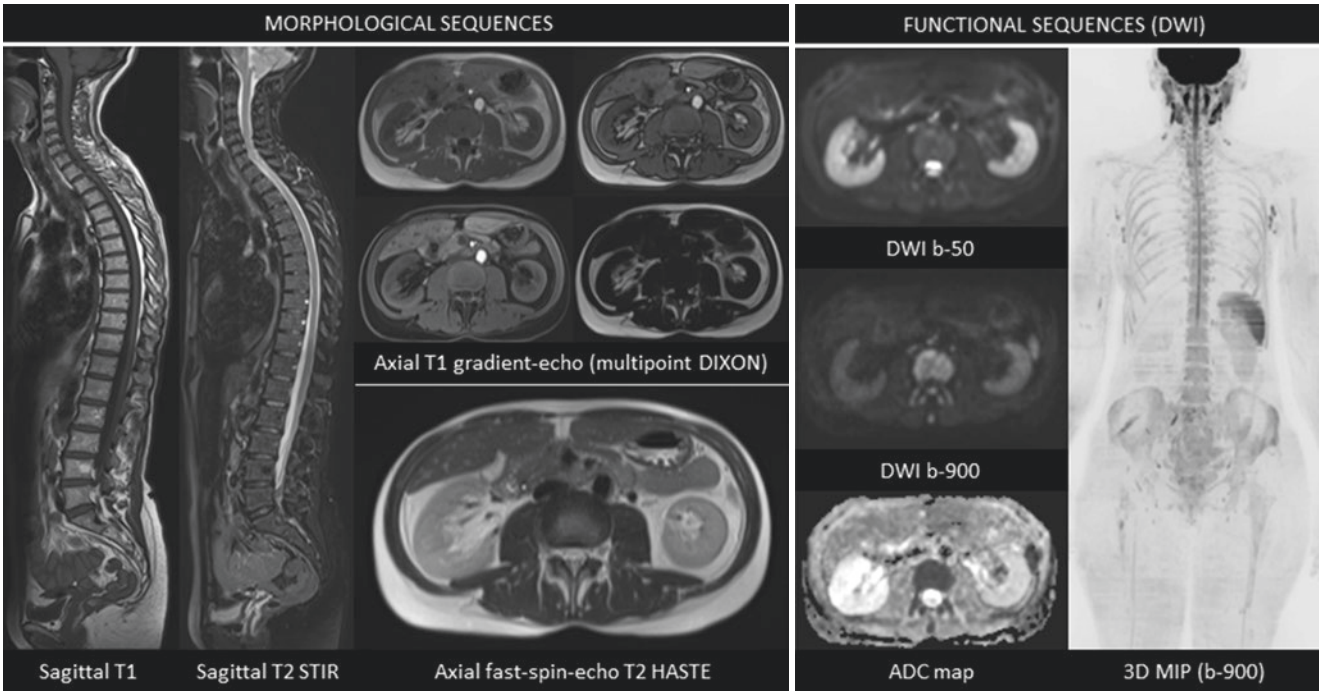

Fig. 20.3 Diffusion whole-body MRI consisting of sagittal T1-weighted and T2-weighted sequences on the whole spine, axial T1-weighted (multipoint DIXON), T2-weighted and diffusion-weighted images from head to mid-thigh, performed on a 1.5 T scanner (Magnetom Avanto, Siemens Healthcare Sector, Erlangen, Germany). Anatomy-specific phased-array surface coils are used for all body regions. The images are processed on a dedicated workstation (Leonardo, Siemens Healthcare Sector, Erlangen, Germany) to produce a unified axial series covering from head to mid-thigh and greyscale apparent diffusion coefficient (ADC) maps. Maximum intensity projections (MIPs) around the crania-caudal axis are generated from axial b900 s/mm^2 series and displayed in an inverted greyscale

Possible causes of increased skeletal signal intensity on high b-value images that lead to false-positive findings include bone marrow oedema caused by trauma [41], degenerative joint disease, bone infarction, infection and haemangiomas, isolated red bone marrow islands within yellow marrow and patchy bone marrow hyperplasia due to bone marrow growth factors. It should be noted, however, that a reader's experience, consideration of ADC values corresponding to hyperintensities on high b-value images and reference to the morphologic T1- and T2-weighted MR images can help reduce false-positive findings. Possible sources of false-negative findings include metastatic lesions in the anterior ribs and within the sternum that are sometimes relatively less conspicuous than lesions found in the spine and paraspinal regions; at these sites, respiratory motion contributes to signal losses on high b-value images. Other causes of false-negative results in bone marrow tumour detection include low levels of tumour infiltration (myeloma or densely sclerotic metastases), location of metastases in the skull vault and skull base (due to the adjacent high signal intensity of the brain) and the development of metastases within hypercellular bone marrow. As a general rule, lytic bony metastases are better seen than pure sclerotic metastases because of the lower water and cellular content of sclerotic and treated lesions [42, 43].

A recent review showed that DWB MRI has overall equal performance to FDG-PET for detecting primary tumours and soft tissue metastases [44]. In addition, it has been established that the diagnostic performance of DWI in combination with conventional non-contrast T1- and T2-weighted imaging in detecting liver metastases (the second most common site of metastases) is high, comparable with contrast-enhanced MRI [45]. DWI has also shown good performance for lymph node assessment, as well as for detecting peritoneal/GI involvement, which are other common sites of metastases for breast cancer patients [46]. Thus, DWB MRI is indicated in all breast cancer patients, who need accurate staging of the entire body, including those at high risk of metastases at presentation (inoperable locally advanced breast cancer (T3/T4) patients and those with inflammatory cancer) or with early locoregional relapse. Another emerging application is the use of DWB MRI in pregnant women with breast cancer [47]. Due to their lowered immunity, diagnosis of advanced stage disease is 2.5 times more likely in these patients than in the general population [48], demanding for an accurate bone and liver staging. It is obvious that the absence of contrast agent and radiation exposure makes a DWB MRI the technique of choice in such patients. Finally, DWB MRI is increasingly used in breast cancer women below 35 years of age, to replace bone scan and abdominal-

pelvic CT scan, to avoid radiation exposure. It is well known that the estimated lifetime attributable risk of death from cancer dramatically increases in patients undergoing CT examinations prior to 35 years of age [49]. DWB MRI is often used when equivocal findings are observed with other techniques where non-FDG avid metastases may be present. Moreover, the other most common sites of metastases (after bone) in lobular breast cancer patients are GI organs, peritoneum and pleura; these sites that are difficult to evaluate with PET/CT, CT or ultrasound. Due to the high tissue contrast between hyper-cellular metastases and the suppressed background tissue, DWB MRI may facilitate detection of metastases in these sites (Fig. 20.4).

20.4.4.3 Monitoring Therapy Response

In the context of therapy monitoring, the attractions of DWB MRI are largely those mentioned above in regard to the absence of radiation and contrast agent with the added consideration that in the course of serial imaging, multiple episodes of radiation exposure are avoided. The absence of contrast agent administration in the majority of applications makes DWB MRI extremely useful in patients with impaired renal function, as well as helping to prevent gadolinium accumulation in the brain [50].

Therapy assessments with DWB MRI are largely made by observing changes in the volume and symmetry of signal-intensity abnormalities on high b-value images, together with changes in ADC values. Nonetheless, correlating the diffusion-weighted imaging findings with morphological appearances on conventional MR images (T1W, fat-saturated T2W/STIR and Dixon) remains important. The lower spatial resolution of diffusion-weighted images is not a real issue in daily practice, as accurate measurements of soft tissue lesions can be easily performed in corresponding axial T1- and T2-weighted images using RECIST or WHO criteria [23, 24]. Although monitoring of bone and soft tissue metas-

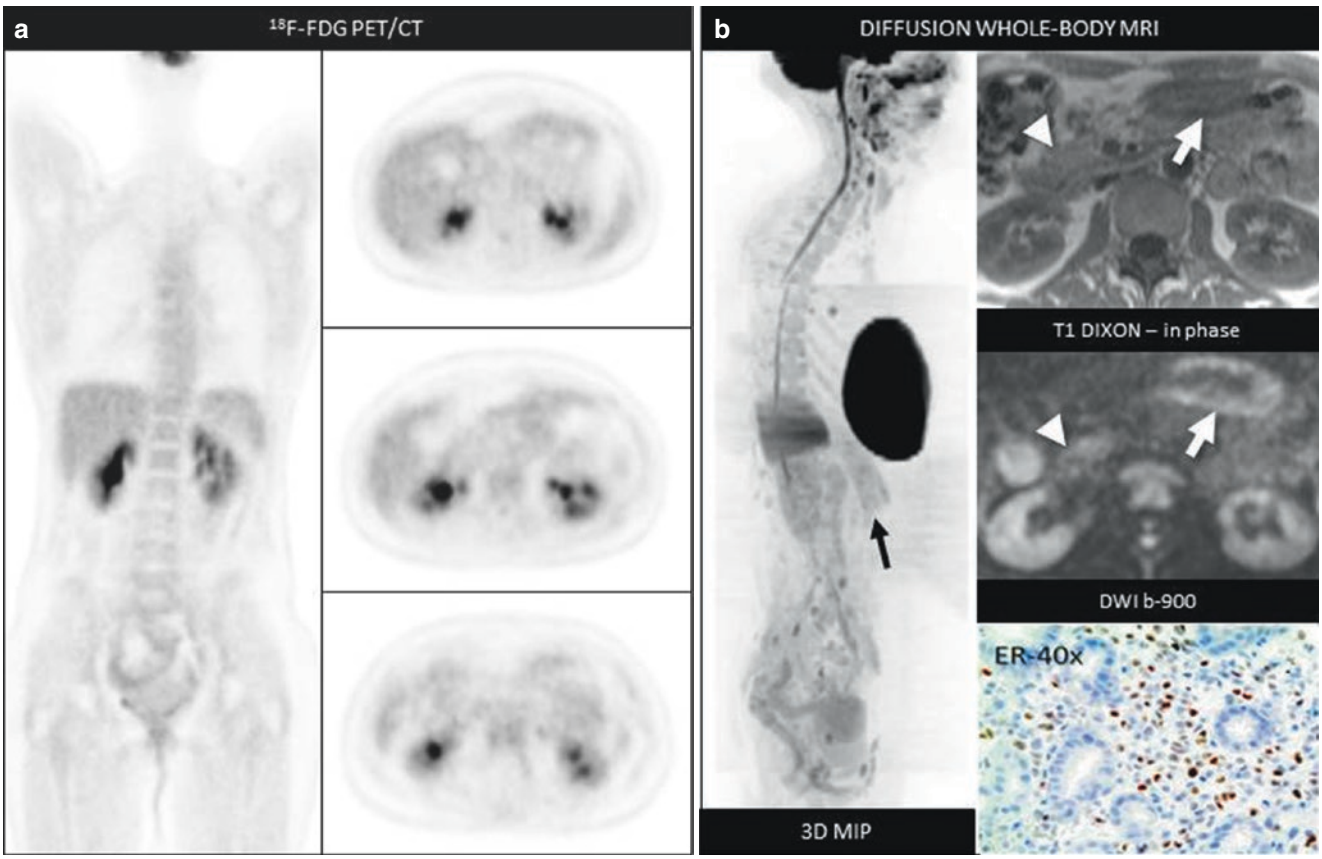

Fig. 20.4 Low sensitivity of 18F-FDG PET/CT for metastases from lobular breast cancer. A 44-year-old woman with operated lobular breast cancer is re-staged with 18F-FDG PET/CT due to suspicion of recurrence after progressive rise in CA 15.3. The FDG/PET examination was negative. After further rises in tumour markers, a second PET/CT (**a**) is performed 8 months later, confirming the absence of detectable disease. DWB MRI is performed at the same time point (**b**), with findings suspicious for the presence of abdominal metastases. Suspicious solid tissue on the right anterior renal fascia is visible both on the anatomical and diffusion-weighted sequences (*arrowheads*). In addition to this, in the DWI sequences abnormally high signal can be seen in the gastric walls (*arrows*): an anatomical site where signal is usually suppressed in b900 images. A second DWB MRI performed two months later confirms these findings. The patient undergoes gastroscopy and multiple punch biopsies of the gastric wall are taken, with positive results for the presence of infiltrating breast cancer (Image of the gastric infiltration for courtesy of Dr. G. Renne, IEO, Milan)

tases is generally based upon similar principles, the evaluation of changes in the bone is unique to DWB MRI, which involves a more detailed and specific process of image interpretation and analysis.

For the monitoring of bone metastases, the lesion-by-lesion signal intensity and ADC value changes can be interpreted using the guidance in Fig. 20.5 with several distinct patterns being recognized in the therapy assessment setting [51].

When bone metastases are treated successfully, the death of tumour cells results in cellular membrane destruction and liberation of intracellular water, which results in increases in water diffusivity, manifested as higher ADC values [52, 53] (generally above the threshold of 1400–1500 μm²/s). ADC increases may be greater for therapies that result in tumour cell death via necrosis rather than via apoptosis because of the associated inflammatory response [54], but this has not been definitively shown. As we have already noted, a prominent response mechanism is the development of dense osteoblastic lesions (osteoblastic scar), the sclerotic response category in Fig. 20.2. Regardless of the mechanism of tumour cell death, in the majority of lesions responding to therapy, high b-value images tend to show signal decreases (Fig. 20.6). Occasionally, however, a successful response to therapy with marked rise in ADC values may yield little change in high b-value signal intensity changes due to T2 shine-through (Fig. 20.7).

When bone metastases are not treated successfully, an increase in the volume of previously documented abnormal signal intensity on high b-value images is observed with new areas of abnormal signal intensity. Increases in the intensity of abnormalities on high b-value diffusion-weighted images can also indicate disease progression (Fig. 20.8).

Importantly, bony metastases that progress can have variable changes in ADC values, with modest increases, unchanged or slight decreases in ADC values compared to pre-therapy values that can occur [52, 55]. Reductions in

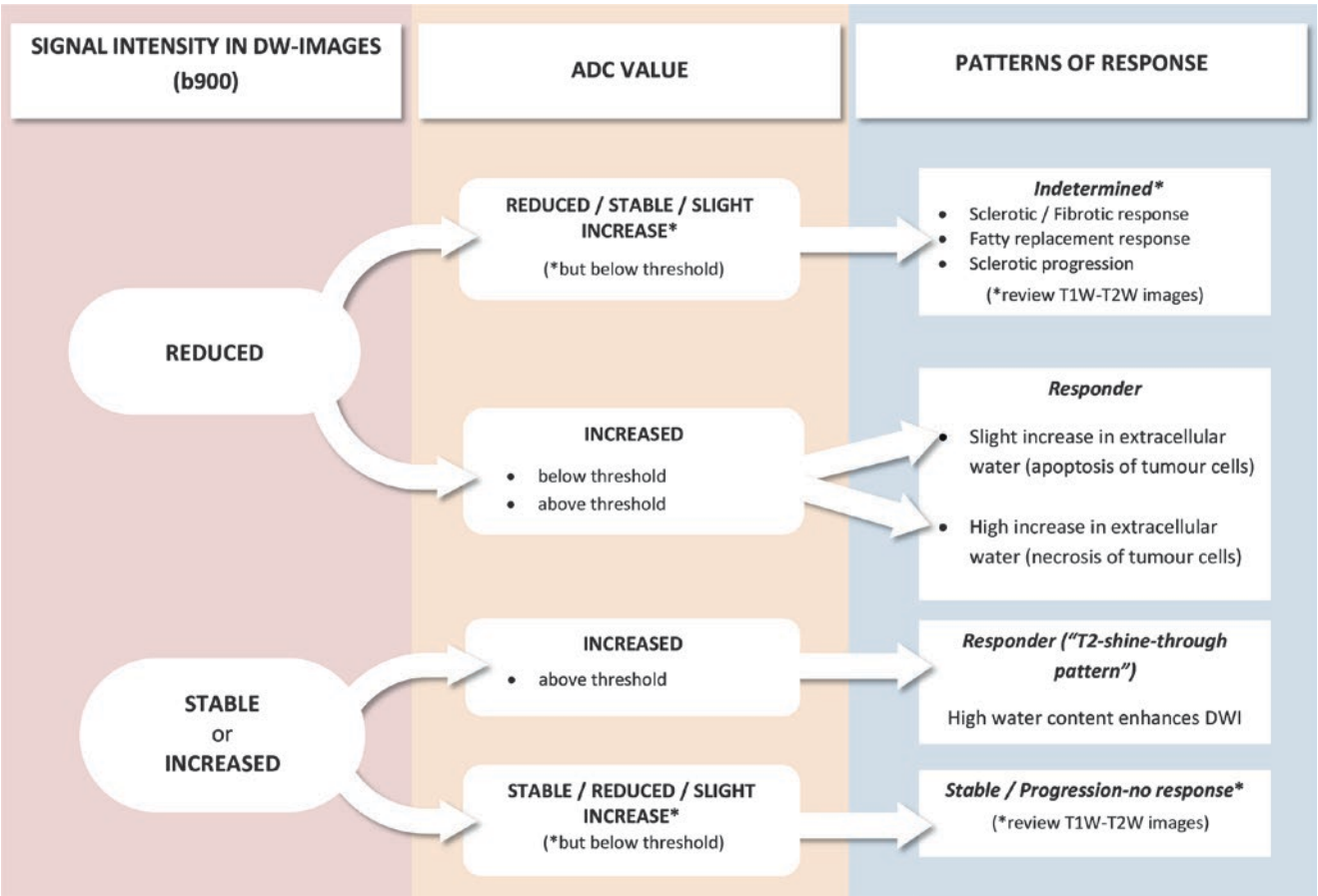

Fig. 20.5 Proposed scheme for assessing therapy response of bone metastases using diffusion-weighted MRI scans, ADC measurements and morphologic images [adapted from *Therapy Monitoring of Skeletal Metastases with Whole-Body Diffusion MRI*; Padhani AR et al. J Magn Reson Imaging 2014]

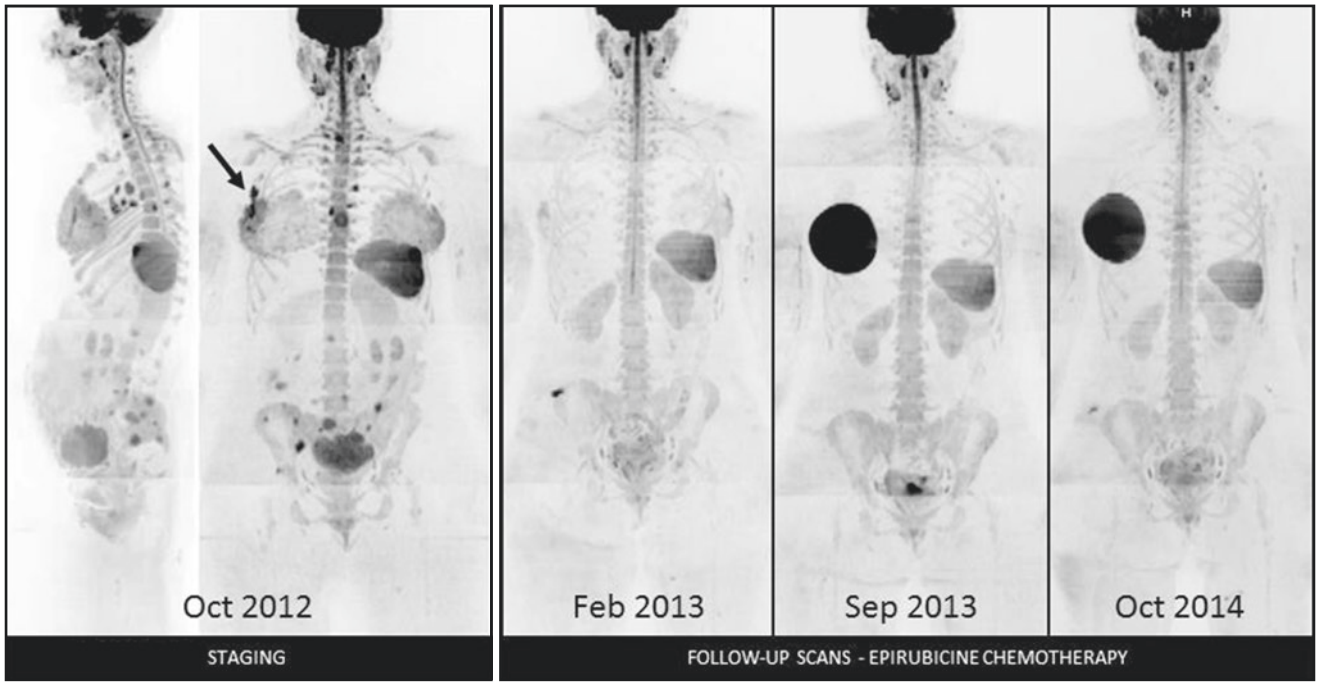

Fig. 20.6 Disease staging and follow-up in a 31-week-pregnant woman with newly diagnosed breast cancer. A pregnant 37-year-old woman is diagnosed with infiltrating breast cancer after fine-needle biopsy of a right breast solid lesion. DWB MRI at staging purpose (October 2012) revealed bone metastases in the dorsal and lumbar spine, in the pelvis and in the sternum. All of the lesions are visible in the rotational b900 MIP reconstruction. The brain, spinal cord and the kidneys of the foetus and mother are well visualized. The right primary tumour and right axillary lymph node enlargement can be seen (*arrow*). Chemotherapy was started after caesarean section. Follow-up scan on February 2013 after three cycles of chemotherapy shows complete response of the bone lesions, with loss of signal on b900 DWI images. All following DWB MRI follow-up scans confirmed a sustained, complete response to chemotherapy

ADC values are probably related to increasing cellularity within a fixed bone marrow space or due to bone sclerosis. Stable ADC values could occur with unchanged tumour cellularity accompanying increases in the geographic extent of disease. The causes for modest increases in ADC values with disease progression are related to increasing tumour infiltration, which displaces fat cells, increases bone marrow water (including water in the extracellular space) and increases tissue perfusion, thus returning higher ADC values compared to yellow or mixed bone marrow [42, 56–60]. ADC values in excess of 1400–1500 $\mu m^2/s$ are rarely seen with disease progression unless there is de novo tumour necrosis.

Further developments of DWB MRI include the quantitative tumour volume assessments that can be undertaken by segmenting high signal intensity regions on high b-value images. Corresponding whole-body ADC histograms can also be generated. Improved precision of response assessment can be undertaken by deriving "viable tumour volume" using threshold ADC cut-off values to exclude normal bone marrow (<600–650 $\mu m^2/s$) and non-viable necrotic tumour with ADC >1400–1500 $\mu m^2/s$ [42, 58]. The proportion of viable tumour can then be calculated and followed over time in the subsequent DWB MRI examinations.

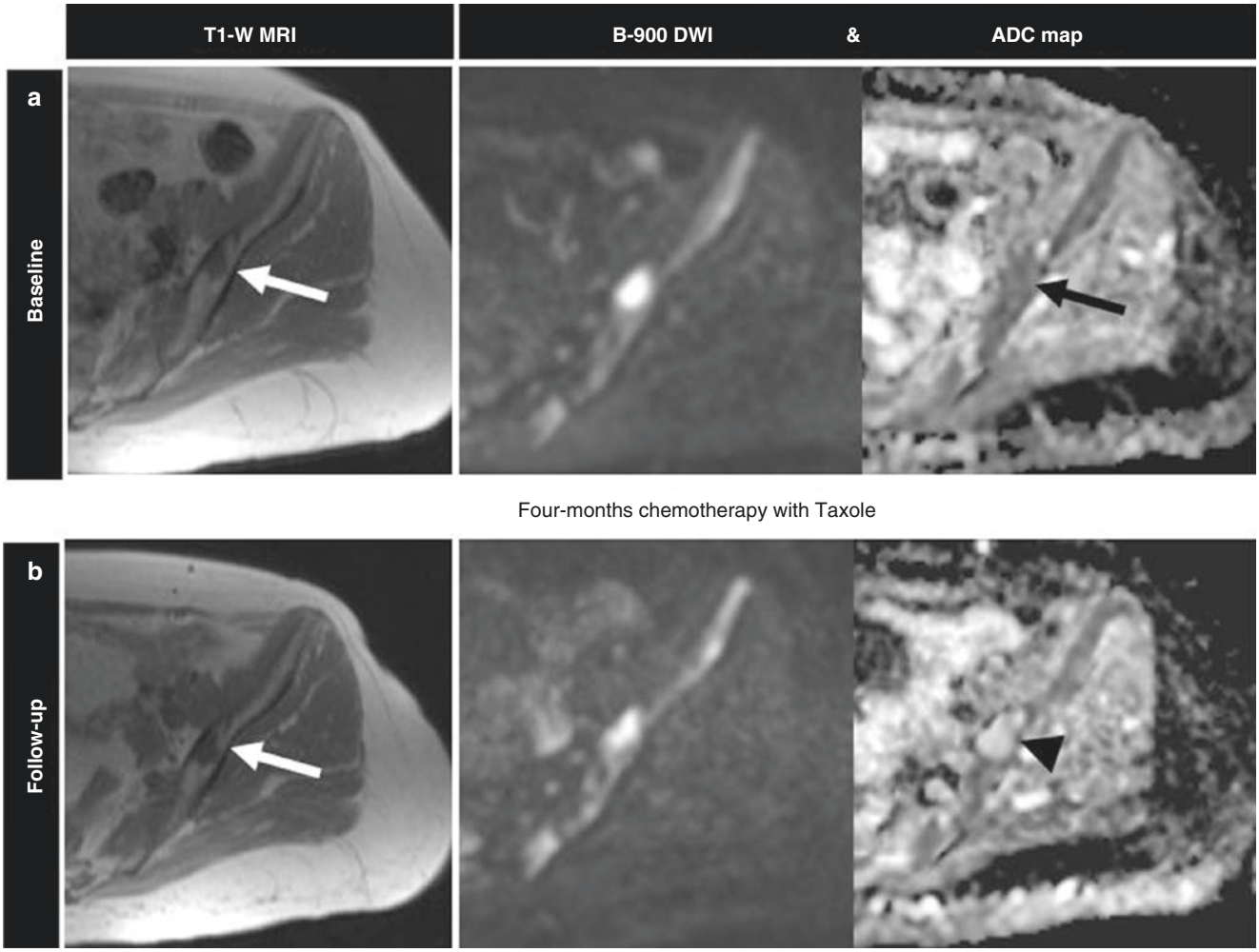

Fig. 20.7 T2 shine-through pattern indicating successful response to chemotherapy. A 51-year-old woman with bone-metastatic breast cancer undergoes DWB at baseline and after treatment with Taxol. (**a**) a metastatic lesion in the left iliac bone is shown as acquired in the baseline evaluation, on T1-weighted and b900 DWI images, as well as on the related ADC map. The lesion has a hypo-intense appearance on T1-weighted images (*white arrow* in **a**) and has a high signal on b900 images with corresponding low ADC values (*black arrow*), suggesting the presence of active disease. (**b**) A second evaluation with DWB after chemotherapy shows unchanged features of the metastasis in the T1-weighted images (*white arrow* in **b**). The lesion maintains high signal in b900 images. The ADC map of the lesion reveals a significant elevation in the mean ADC values (above 1500 $\mu m^2/s$, *black arrowhead*) indicating complete response to chemotherapy. The high signal in b900 images with accompanying high ADC values is termed "T2 shine-through"; the latter is strongly associated with cell necrosis and tumour response

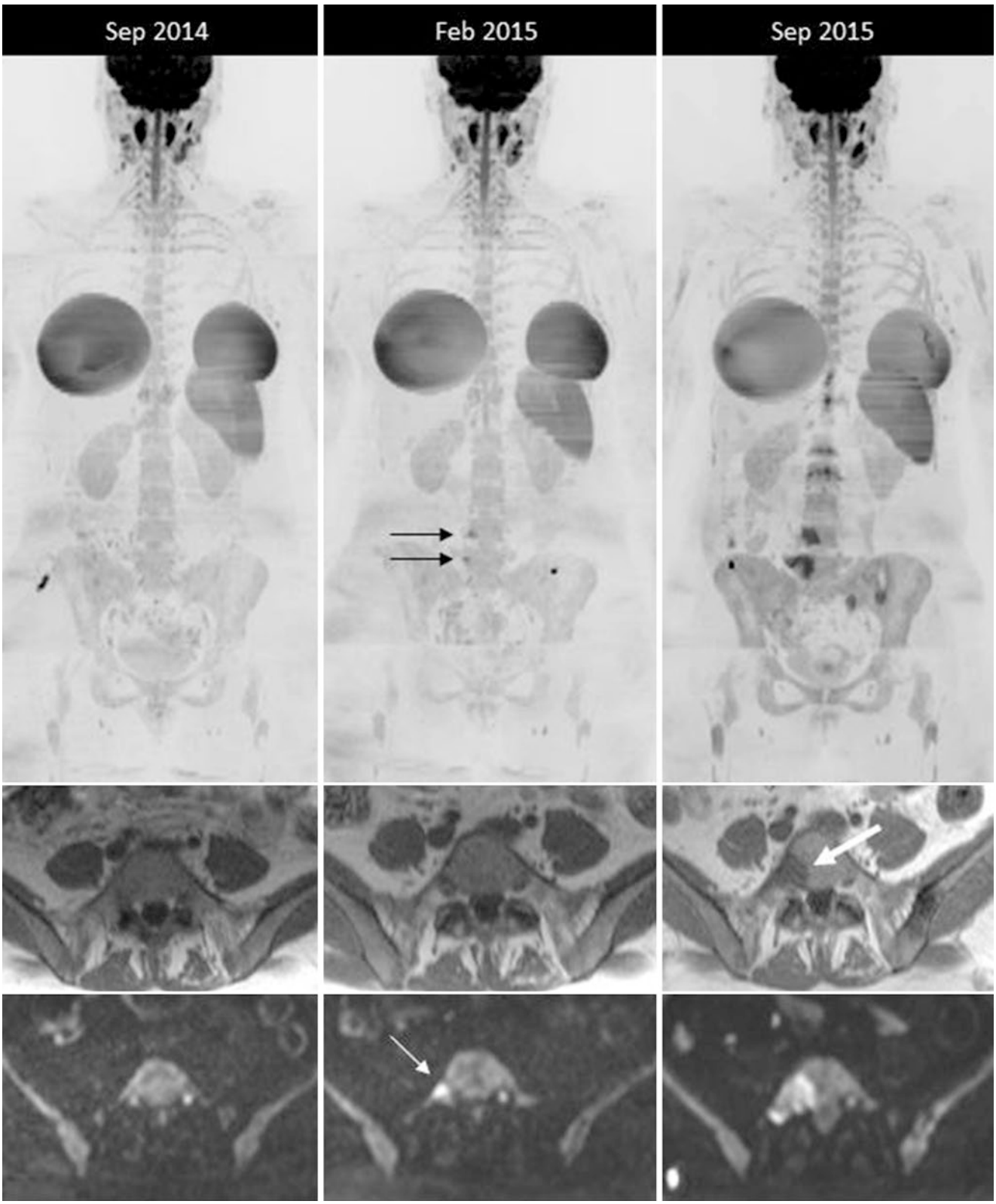

Fig. 20.8 Bone disease progression in anatomical and diffusion-weighted images. Serial changes in a 39-year-old woman with progressive metastatic breast cancer being treated with hormonal therapy and bisphosphonates. Axial anatomical (T1W) and DWI images (b900) and inverted coronal MIP images allow bone metastases to be evaluated over time. The second scan shows bone disease progression with re-activation of lesions not visible in examination 1 (*black arrows*). Increases in the number of lesions in the lumbosacral spine and pelvis are seen on examination 3. Axial images show re-activation and then growth of a bone metastasis located adjacent to the right sacroiliac joint despite therapy change in February 2015. Note the presence of a new lesion posteriorly in the left iliac bone adjacent to the left SI joint whose signal on high *b*-value images is being moderated by osteosclerosis

Conclusions

Whole-body MRI has the potential address the unmet clinical need for an accurate method to detect and monitoring response of all manifestations of metastatic breast cancer. There is a need to develop common measurements and analysis methods and to establish uniform data displays, to expand the use of quantitative analyses of DWB MRI in clinical practice. The technology is now mature enough to incorporate into clinical studies that define appropriate use of this technology.

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Imaging Findings After Surgery

21

Silvia Pérez Rodrigo and Elizabeth A. Morris

21.1 Introduction

Breast cancer treatment has markedly improved in the last few decades. Radical mastectomy was initially considered the treatment of choice despite its associated morbidity. However, less aggressive surgeries such as modified radical mastectomy, simple mastectomy, skin-sparing mastectomy, and breast-conserving surgery have increasingly replaced radical mastectomy. Moreover, reconstructive surgeries such as oncoplastic surgery have entered the surgical scene, combining a safe oncological treatment approach with desirable aesthetic outcomes. Many women being treated for breast cancer have also undergone breast-enhancing procedures using exogenous material that are either heterologous or autologous. These procedures can be performed for purely aesthetic reasons or for reconstructive purposes in cases of breast cancer.

The greatest difficulty in assessing breast tissue postsurgery is to distinguish postsurgical changes from malignancy. Recurrence is possible despite the treatment of breast cancer, including in patients who have undergone mastectomy. Thus it is necessary to know the radiological techniques available to study recurrence in patients and to establish an appropriate follow-up.

Mammography can detect tumors which are then less likely to develop metastases during follow-up. However, mammography has limited sensitivity, from 55 to 68%. There are also difficulties in performing mammography correctly with adequate compression, and the radiologist does not always obtain good image quality with mammography. Mammography also presents difficulty in interpretation, for example, in differentiating spiculated scars from recurrence and differentiating edema (due to the RT) from lymphatic involvement. A clinical examination complements mammography in detecting recurrences, and a palpable lesion usually indicates worse prognosis.

Compared to clinical examination and mammography, MRI has high sensitivity, from 90 to 100%. It also has high specificity, from 83 to 93%. It is also able to reliably distinguish scar from recurrence 12 to 18 months posttreatment. Breast MRI allows both morphological and functional evaluation. Non-enhancement (absence of uptake) after intravenous contrast (IVC) suggests fibrosis rather than cancer. However, the presence of enhancement does not always indicate malignancy because there are many benign processes that can cause uptake, such as fat necrosis. Consequently, if the presence of fat inside the lesion cannot be determined with fat suppression sequences, a core biopsy is required.

Ultrasound has limited sensitivity for small or noninvasive lesions. Acoustic posterior shadowing on ultrasound presents difficulty in differentiating fibrous scar from recurrence.

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21.2 Imaging Findings After Surgery Without Any Material or Reconstruction

21.2.1 Mastectomy

An estimated 10–15% of patients will develop locoregional recurrence after treatment. Nearly a third of them will present with synchronous metastases at diagnosis. The most common form of presentation (50–70%) is local recurrence (Fig. 21.1a–c), which tends to be symptomatic. The patient has a single mass or multiple masses in the bed of mastectomy (chest wall or under the scar), diffuse skin thickening, trabecular thickening, or ulceration of skin. Recurrence can manifest radiologically as masses with signs of suspicion and/or calcifications

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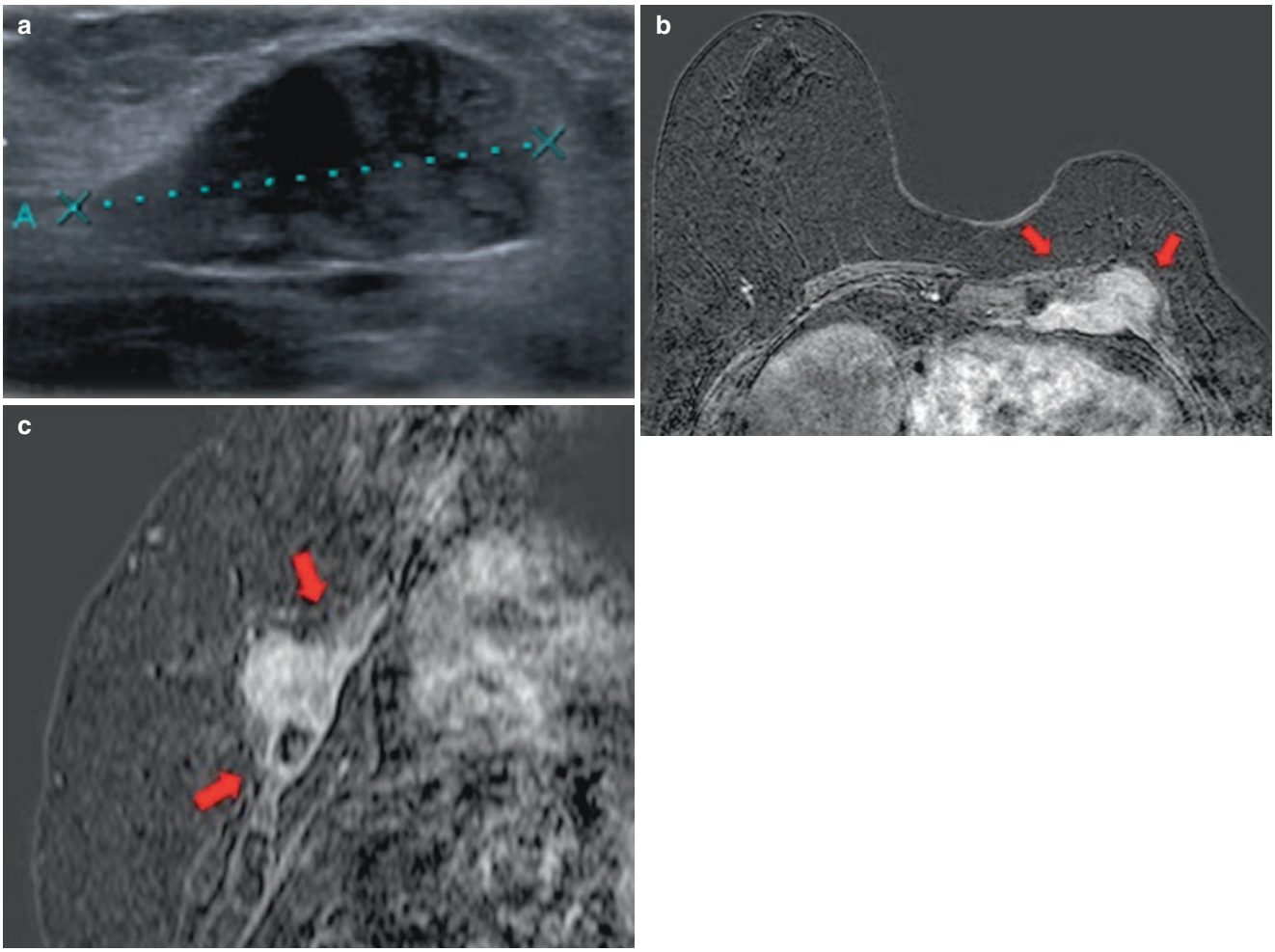

Fig. 21.1 Patient with history of left mastectomy noticed a palpable lump in the mastectomy site corresponding to recurrence. (**a**) Ultrasound: an anechoic and heterogeneous lesion, ill-defined in some margins and seeming to be in contact with the pectoral muscle, is seen. (**b**) Breast MRI (dynamic sequence, axial plan): a mass in the mastectomy site, involving the pectoral muscle, some ribs, and intercostal muscles and goes inside the thoracic cavity, is seen. (**c**) In the sagittal view, involvement is well seen in the costal and intercostal spaces

that in some cases can be difficult to distinguish from dystrophic calcifications or fat necrosis. Recurrence can also present in regional or distant lymph nodes (30–40%), which are usually asymptomatic (Fig. 21.2) and may not be palpable, at least initially. Some common locations are the supraclavicular, axillar, and internal mammary regions, where they can cause pain, brachial plexopathy, or arm lymphedema. Any new onset lymphedema after treatment should be evaluated to rule out regional recurrence.

21.2.1.1 Radiological Tests and Findings

1. *Mammography*: Mammography is not routinely performed for patients who have undergone mastectomy. However, it can be performed, and the mammogram can be examined for the presence of residual breast tissue, especially in cases of subcutaneous or incomplete mastectomy.

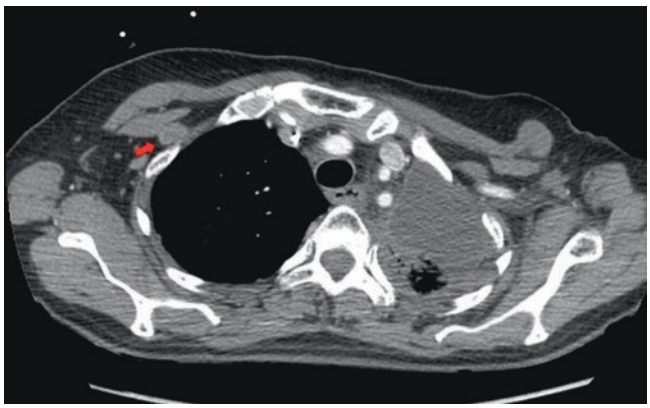

Fig. 21.2 Patient with history of left mastectomy. CT axial plane shows an enlarged and round retropectoral lymph node, suggesting recurrence. Despite retropectoral involvement, the axillary lymph nodes were negative and asymptomatic

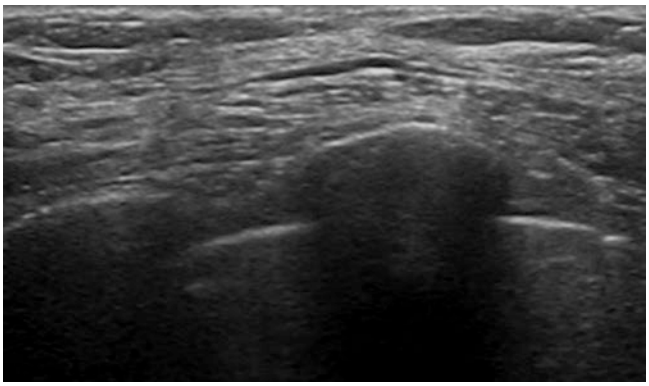

Fig. 21.3 Sonographic appearance of a mastectomy. The skin, subcutaneous tissue, and the pectoral muscle on the chest wall can be appreciated

2. *Ultrasound*: Patients who have undergone mastectomy may be examined by bed ultrasound. Ultrasound allows the physician to observe subcutaneous fat, postsurgical scarring, and fibrosis (Fig. 21.3). Nodular lesions on ultrasound suggest recurrence (Fig. 21.1a), and as patients undergoing mastectomy do not routinely undergo mammography, these should be evaluated carefully. Possible complications or benign findings such as seromas or hematomas should also be assessed.
3. *CT or MRI*: Postsurgical changes in the axillary level and the presence of residual breast tissue should be evaluated. The type of surgery and the presence or absence of the pectoral muscle should also be noted before the interpretation of findings.

21.2.1.2 Follow-Up Protocol

In the follow-up of patients, physical examination and ultrasound are usually performed. Mammography is performed only in cases where incomplete mastectomy or residual breast tissue is suspected and when technically feasible (Fig. 21.4). Breast MRI can be performed to show the absence of residual glandular tissue (Fig. 21.5). It has shown greater sensitivity and specificity than other techniques; however, it is rarely used routinely due to its availability and cost.

21.2.2 Breast-Conserving Surgery

There is a risk of recurrence in about 1–2% patients post-breast-conserving treatment per year. Early detection is crucial because it is associated with improved survival. Presentation of recurrence can manifest early or late. Early recurrence usually occurs at the site of the original tumor and represents failure in the eradication of the primary tumor (Fig. 21.6a, c, and d). Late recurrence (after 10 years of

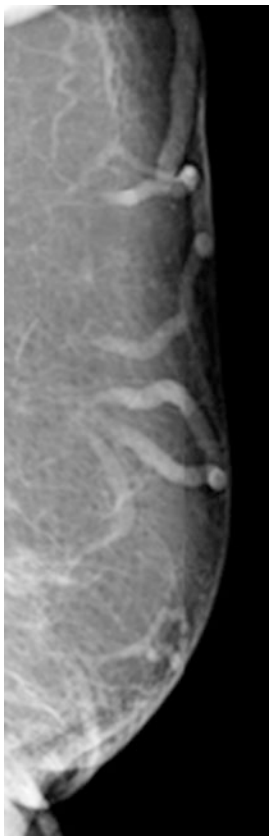

Fig. 21.4 Patient with left mastectomy. Mammography (oblique view) was performed to rule out the presence of residual fibroglandular tissue

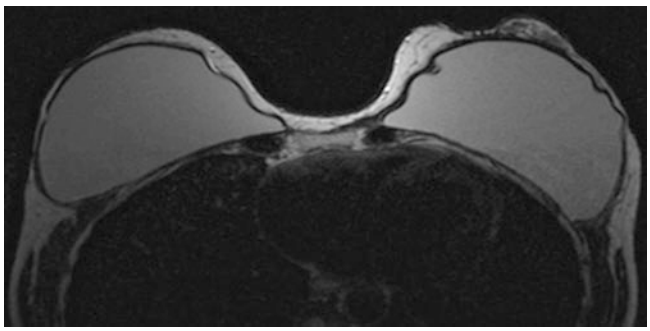

Fig. 21.5 35-year-old patient with a history of right breast cancer and positive BRCA1. Right mastectomy and prophylactic left mastectomy were performed. The patient was not a candidate for mammography. Breast MRI was performed to check whether there was residual fibroglandular tissue. T2 sequence shows the presence of residual fibroglandular tissue with slight enhancement on the left breast. A follow-up MRI was recommended

finishing the treatment) usually occurs in another quadrant or away from the treated area and usually represents new tumors.

Interpretation of imaging post-breast-conserving surgery can be complicated because postsurgical benign changes can simulate recurrence and make the follow-up overwhelming. Early recurrence usually occurs after 2 years following

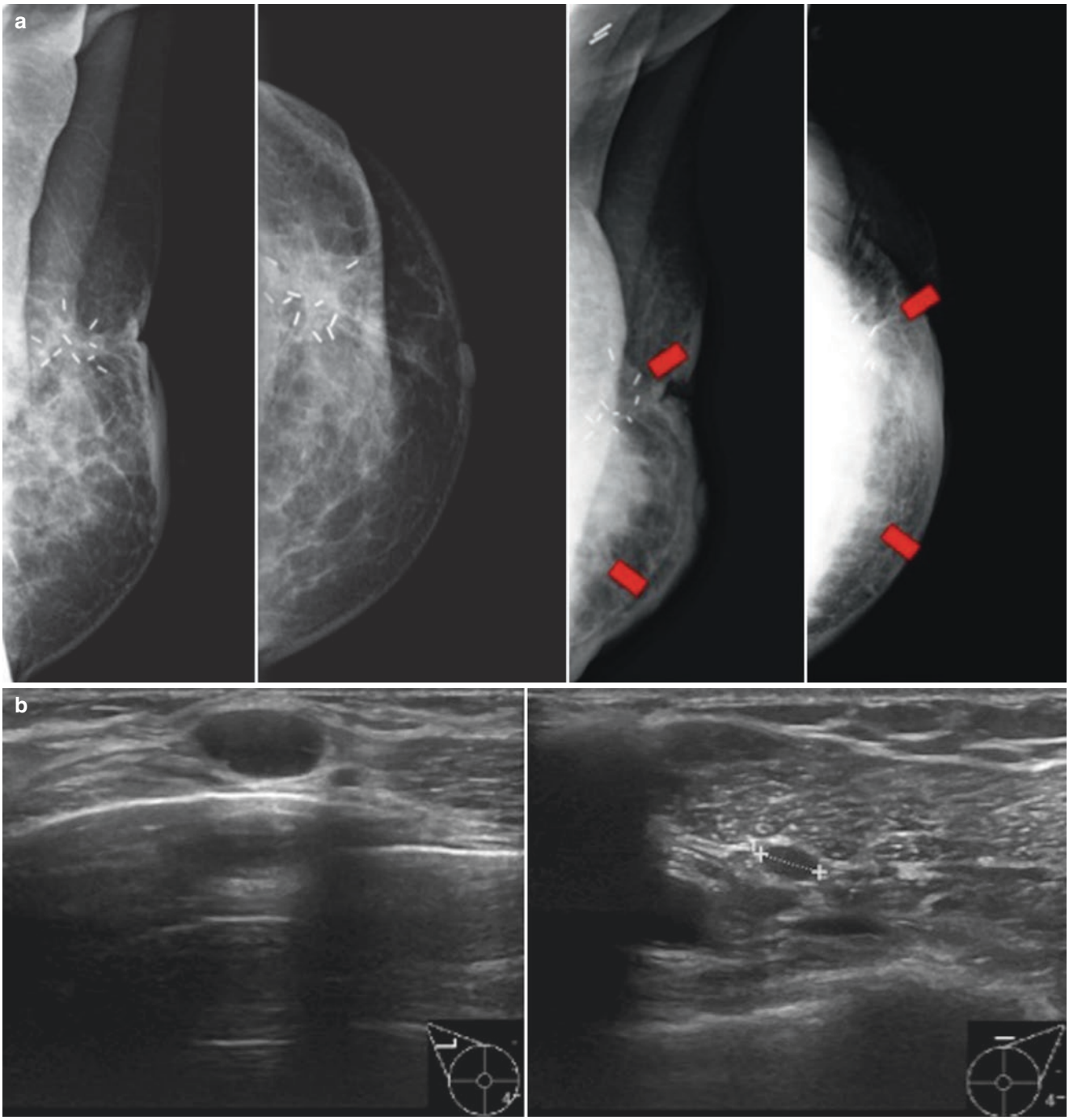

Fig. 21.6 Patient with triple negative breast carcinoma in the right UOQ and axillary involvement. The patient received neoadjuvant chemotherapy. MRI showed a complete response. Therefore, breast-conserving surgery was performed. After the surgery, the pathologist reported positive margins, and the patient received radiotherapy but was not reoperated. (**a**) Mammography with CC and oblique views: On the left, first mammogram after surgery shows a distortion relating to post-surgical changes adjacent to the clips. On the right, second follow-up mammogram shows a diffuse increase of density involving the pectoral muscle, in comparison with the previous mammogram, suggesting recurrence (*red lines*). Skin thickening was also noted. (**b**) Ultrasound confirmed these findings where new involvement of axillary and infraclavicular lymph nodes as well as contralateral axillary lymph nodes is also seen

treatment, whereas during the first 2 years, most radiological changes are due to benign changes. To distinguish recurrence-related and benign changes, radiologists need to be aware of the following expectations. Benign changes should decrease or remain stable over time. Stability is usually achieved 2–3 years after RT. After this, any postsurgical changes, density or new onset suspicious calcification, should rule out recurrence (Fig. 21.7).

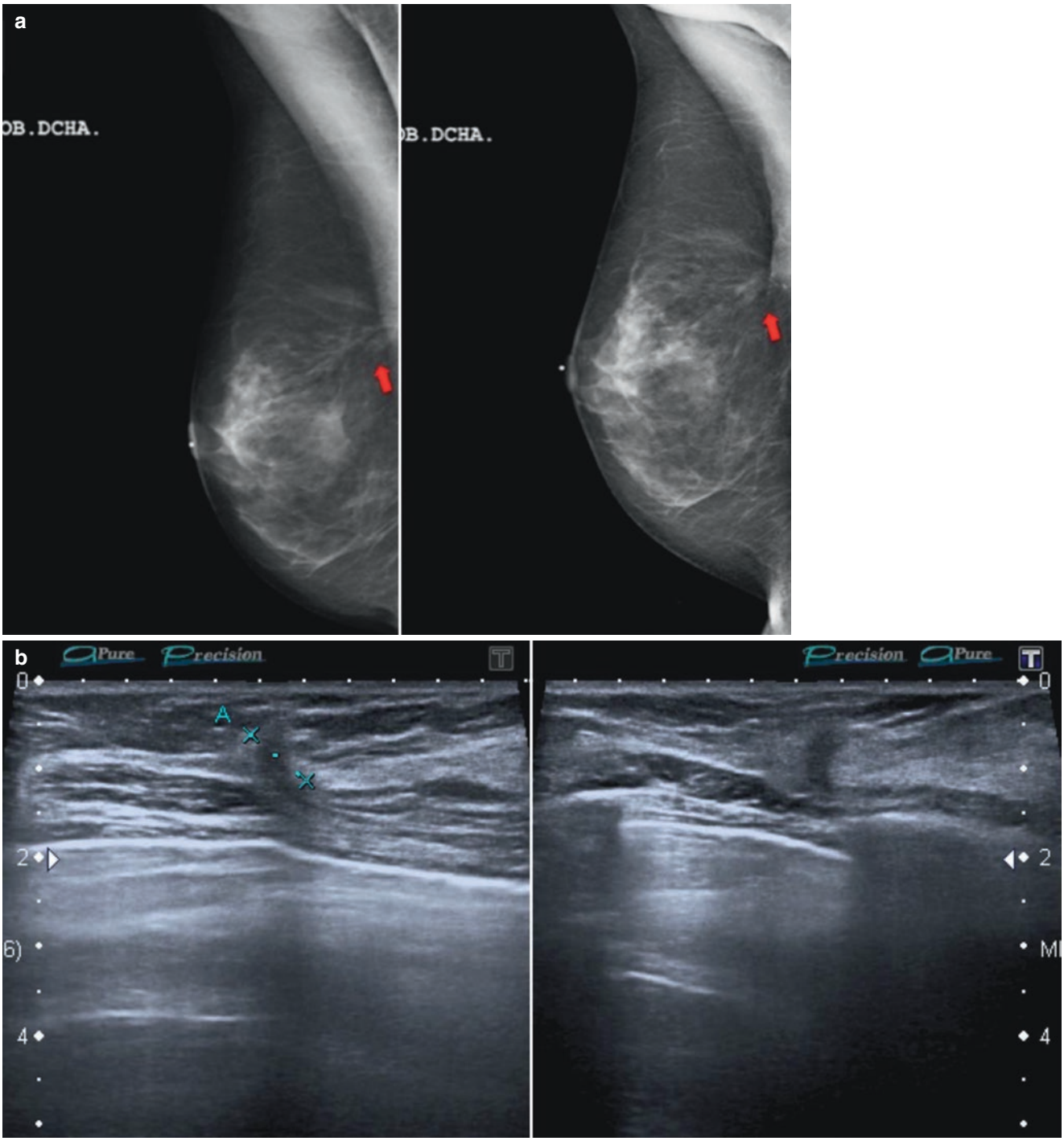

Fig. 21.7 Patient with breast-conserving treatment in the UOQ of the right breast. (**a**) Mammography oblique views: On the left, a follow-up mammogram a few years after surgery demonstrates subtle and stable distortion in the postsurgical area (*red arrow*). On the right, another mammogram a year after shows the distortion presenting a slight increase in density. An ultrasound was recommended. (**b**) A hypoechoic, ill-defined and irregular lesion can be seen with an antiparallel orientation suggesting malignancy that was confirmed with a core needle biopsy

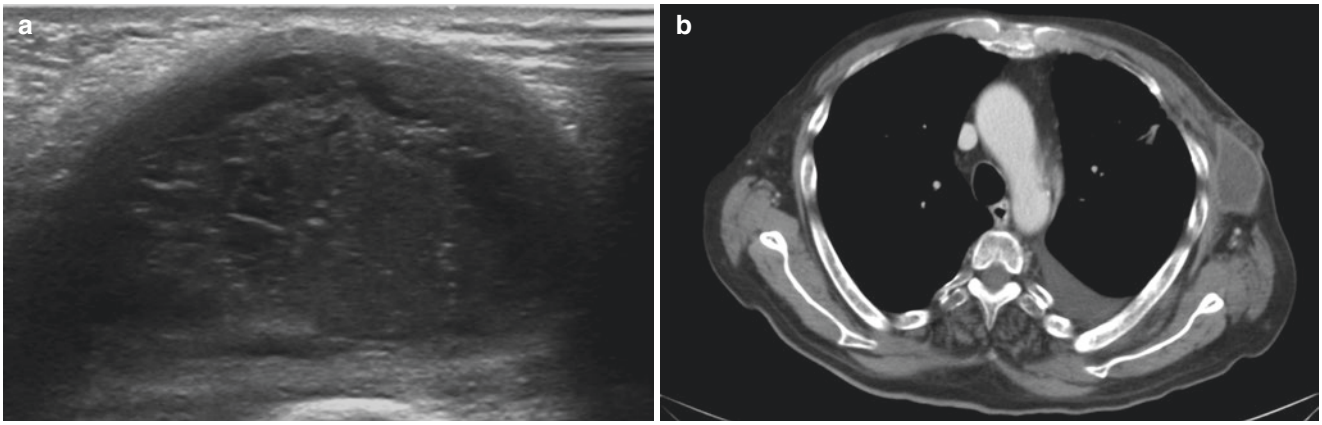

Fig. 21.8 Patient with a personal history of left mastectomy and axillary dissection. The patient showed a palpable lump in the axillary region corresponding to an evolved seroma/hematoma without changes in follow-up. In normal conditions, the seroma or hematoma is reabsorbed a few times after surgery. In this case, it did not happen although it was stable. (**a**) Ultrasound of the axillary region shows a complex lesion with internal septa and a thick wall relating to the palpable lesion. (**b**) CT shows fluid collection with different densities and thin enhancement of the wall

21.2.2.1 Radiological Tests and Findings

The following are considered benign findings after breast-conserving surgery:

Seroma/hematoma is present when there is fluid collection in the surgical area. In most cases, over time, seroma/hematoma will be reabsorbed and replaced by fibrosis and scarring. Fifty percent of the cases will last a month after surgery, 25% will last 6 months after surgery, and most of them will disappear within 12–18 months after surgery. Sometimes seroma/hematoma may persist over time (Fig. 21.8):

- *On mammogram*: Seroma/hematoma will appear as a highly dense, oval, or round mass. It can have well-defined margins or obscured margins when it lies within the distortion produced by benign postsurgical changes.
- *On ultrasound*: Seroma/hematoma will appear as an anechoic collection normally seen as a simple cystic lesion. Over time, seroma/hematoma will decrease in size and have increased echogenicity showing a complicated or complex appearance (septa, loculations, and/or thickening of the wall).
- *On CT and MRI*: Fluid collection will be seen in the surgical site. Water density and signal intensity will vary over time according to cancer stage.

Edema and skin thickening will be most highly expressed 6 months after RT and later evolve and regress in a similar way. The edema may be focal in the area of the lumpectomy (reflecting postsurgical changes) or be distributed diffusely (reflecting changes post-RT). If there is an increase of edema, the physician should perform differential diagnosis regarding tumor to lymph vessels, obstruction of venous drainage, or congestive heart failure and infection. Skin thickening is secondary to the damage produced on small vessels and can measure up to 1 cm in thickness. It is more evident when the treated breast is compared to imaging of the contralateral breast or to its appearance on the pretreatment mammogram (Fig. 21.9). On MRI these findings are well observed, especially on T1 sequence (where we can observe the skin thickening) and T2 sequence (where edema is clearly hyperintense) (Fig. 21.10).

Architectural distortion occurs as a result of changes in scarring and fat necrosis. It reaches its highest expression at 2 years. Thereafter, it will remain stable or decrease. Architectural distortion can have spiculated or irregular density as well as poorly defined margins and may be accompanied by skin retraction; these can resemble recurrence. A number of mammographic findings such as central radiolucency, appearance changes in different projections, and thick and curvaceous spiculations may suggest that the findings are more likely of postsurgical origin. Note that findings are not always reliable; for example, there are some lobular carcinomas that may also present central radiolucency. For this reason, it is essential to perform magnified and spot compressed views (Fig. 21.11) as well as a comparative study with previous mammograms (to verify the stability or progressive reduction in size and density) (Fig. 21.7), and if there is still some doubt, biopsy should be performed (Fig. 21.12).

Benign calcifications usually appear in the first 6–12 months in 28% of cases. They can usually be distinguished from pleomorphic calcifications associated with malignancy, but when there is uncertainty, additional views, tests, or biopsy are required. Benign calcifications can be dystrophic calcifications, which usually originate in areas of fat necrosis. These are irregular in shape, and they are

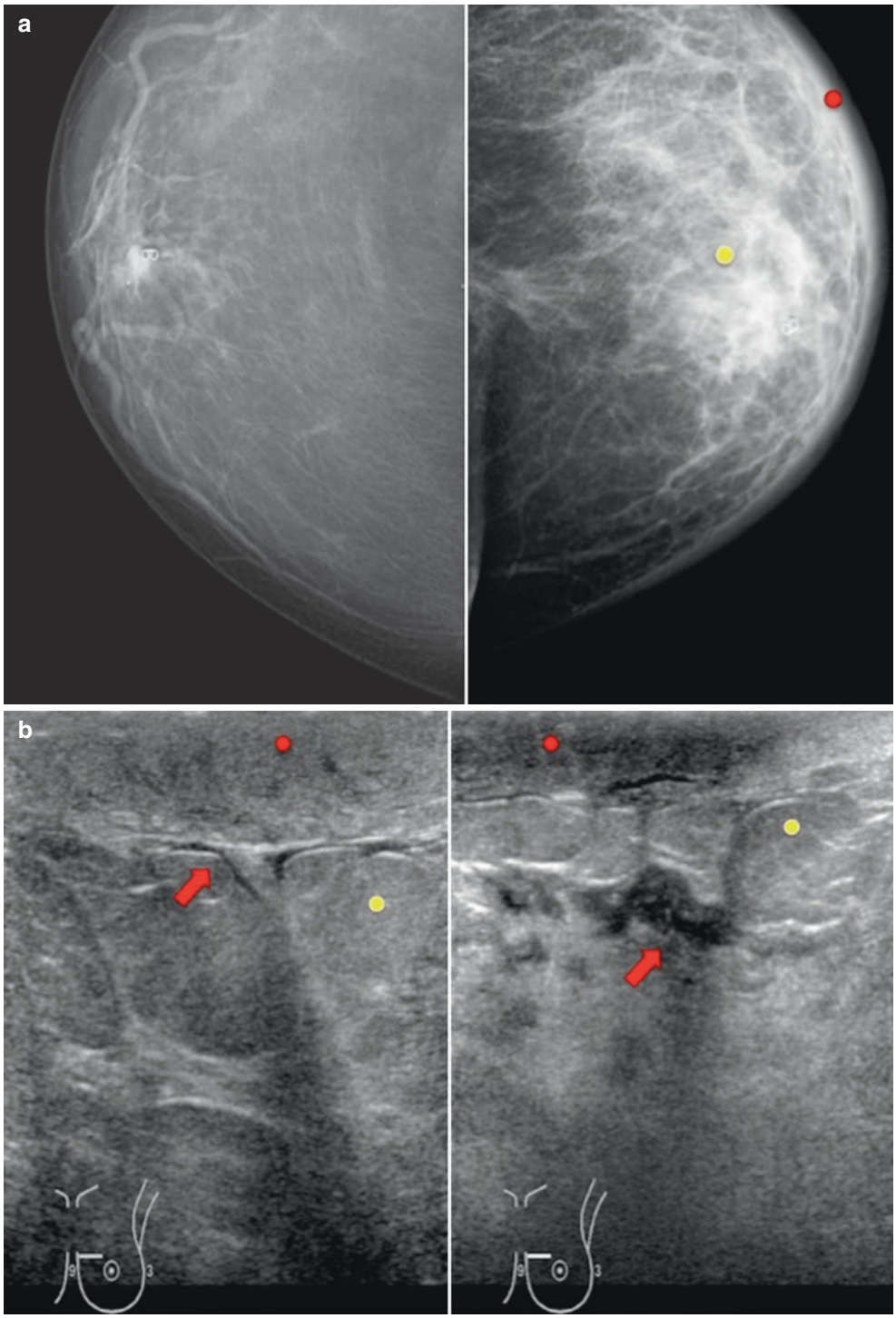

Fig. 21.9 Patient with breast-conserving treatment in the left breast with surgery in LIQ and RT. The patient presented with marked skin thickening with diffuse subcutaneous tissue edema at 6 months after finishing treatment as well as hot, red, and induration in the breast. (**a**) Bilateral mammography CC views show skin thickening (*red dot*), trabecular thickening, and increased density (*yellow dot*) relating to edema and post-radiotherapy changes. (**b**) Ultrasound shows skin thickening (*red dot*), diffuse increased echogenicity relating to edema (*yellow dot*), anechoic linear images between fatty islets relating to distended lymphatic vessels, and an anechoic nodular imaging relating to emerging abscess (*red arrows*). The patient was diagnosed with mastitis. The clinical context is always key because radiological findings of noncancerous entities may mimic breast carcinoma

usually larger than 1 cm. They often have a lucent center. Sometimes they show the typical appearance of rim or eggshell calcification. Benign calcifications can also be suture material, which represent calcium deposited on suture material. They are less common than dystrophic calcifications. They are typically linear or tubular in appearance, and when present, knots are frequently visible.

There is no universal agreement about when to perform the first mammography after finishing RT. In most cases, the first mammography is performed 6 months after RT. In some cases where tumors initially present with calcifications, the initial mammography is performed just before the beginning of RT to verify the absence of residual calcifications.

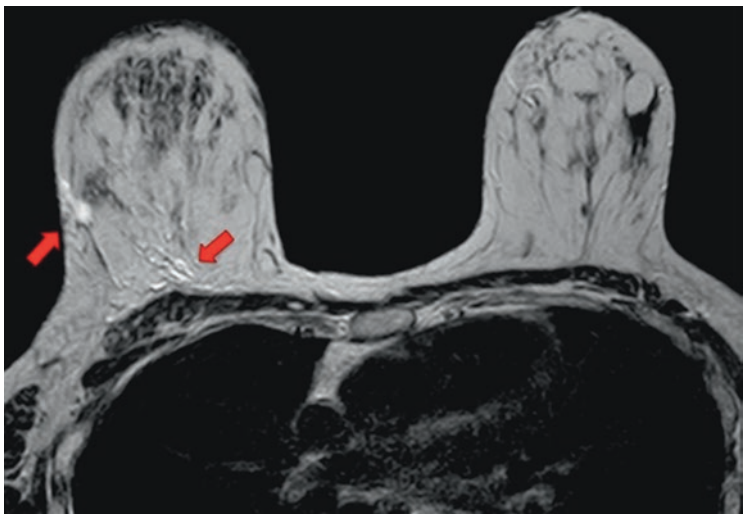

Fig. 21.10 Patient with breast-conserving surgery and subsequent RT treatment in the right breast presented with redness, skin thickening, and fever during her RT treatment. T2 sequence shows hyperintensities regarding the liquid and edema (*red arrow*)

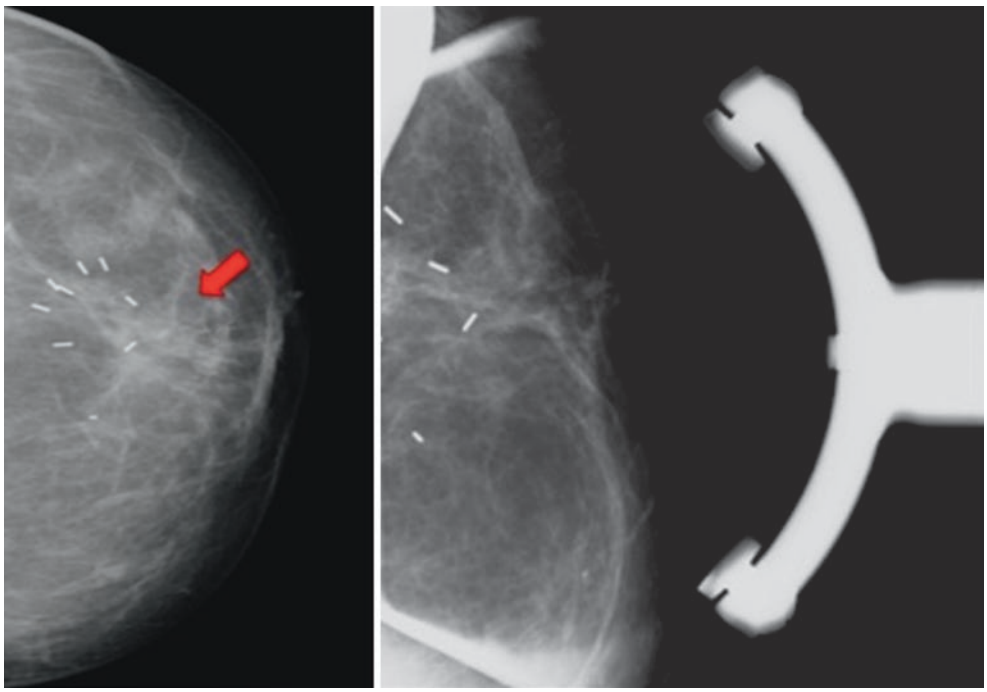

Fig. 21.11 Patient with personal history of breast-conserving surgery in the OUQ of left breast. A distortion image is seen in the area relating to scar tissue. CC view mammography (on the *left*) shows a distortion in the area of the surgical changes. A spot compression view (on the *right*) shows a fat center and dissociation

21.2.2.2 Follow-Up Protocol

A follow-up protocol could be:

- Initial mammography 6 months post-RT (in cases of tumors with calcifications, mammography could be performed just before RT to confirm complete removal of them).
- Annual mammography (± magnified and/or compressed projections) 2 years after RT. This should be considered as baseline as thereafter the posttreatment changes should remain stable or decrease.
- Annual ultrasound with mammography: Optional.

If available, breast MRI is the most useful imaging technique in evaluating cases after breast-conserving surgery because of its high negative predictive value. Nevertheless, if there is any doubt concerning its findings, a histological examination should be conducted.

21.2.3 Mastopexy

For patients who have undergone mastopexy, a new architectural pattern is established which can modify the normal radiological appearance of the breast. These modifications

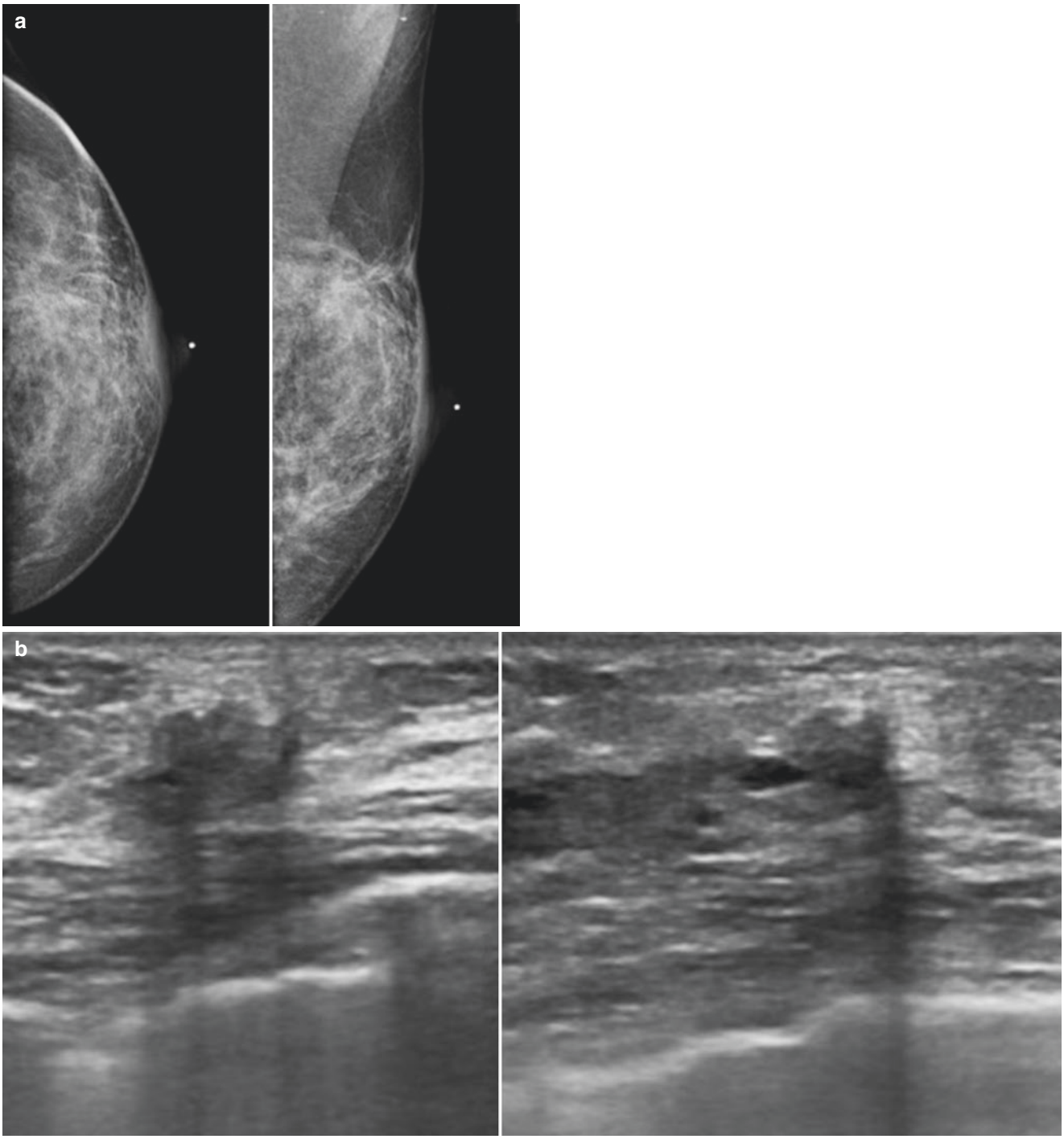

Fig. 21.12 Patient with a personal history of breast-conserving surgery in OUQ left breast. (**a**) Mammography shows architectural distortion in the area of the scar. (**b**) Ultrasound shows a nodular lesion corresponding to the surgical area. Although that image changed with probe position, it was suspicious so a core needle biopsy BAG was performed with result of scarring changes

are due especially to the presence of scars, remodeling and reorientation of breast tissue, and repositioning of the NAC. When mastopexy is performed for cosmetic purposes, postsurgical changes are usually bilateral; therefore, radiological findings are often in both breasts and can be compared. In cases where mastopexy is performed for symmetrization/contralateral reconstruction after mastectomy, radiological findings are asymmetrical and can seem suspicious especially if the patient's imaging history is unknown.

Imaging history should be reviewed to assess possible changes over time. Sometimes important radiological

findings after mastopexy can go unnoticed if the patient's previous history is unknown. Postsurgical findings should decrease over time, and any new or more evident findings should be investigated.

21.2.3.1 Radiological Tests and Findings

1. Mammography:
   - Distortions or focal asymmetries
   - Skin thickening or a dense periareolar line and sometimes in the vertical line that joins the periareolar region with the inframammary fold
   - Benign calcifications—usually skin calcifications, oil cysts predominantly of periareolar location, or coarse calcifications
   - Fibrous bands extending from the repositioned NAC
   - Reorientation of the breast tissue, with higher amount of tissue in the lower quadrants
2. Ultrasound:
   - Shadowing, usually related to scars
   - Heterogeneous areas of breast tissue relating to the repositioning of the gland
   - Ill-defined and heterogeneous lesions when there are fat necrosis changes
3. MRI:
   - New architectural pattern.
   - Cutaneous and subcutaneous postoperative changes, especially in the periareolar region, the vertical scar and the inframammary fold. Sometimes these changes cannot be noticed on mammogram and they can only be detected on MRI. They are more evident on gradient echo and black silicone sequences (Fig. 21.13).
   - If irregular enhancement is seen, differential diagnosis should be done to distinguish fibrosis, fat necrosis, or malignancy, and then, biopsy should be done.

21.2.4 Complications and Sequelae

Complications and sequelae should be considered when interpreting imaging and managing the follow-up of the patient. Seroma/hematoma will disappear in most cases but may persist and be clinically significant in some. In the latter scenario, both increased risk of infection and delayed healing have been shown, so seroma/hematoma may require aspiration or drainage. With cellulitis, skin and subcutaneous thickening and trabecular thickening can be seen. Sonographically they correspond to a diffuse increased echogenicity with hypoechoic septa regarding distended lymph vessels. An abscess (Fig. 21.9b) can occur as a late complication (approximately 5 months after surgery), because of a superinfection of a previous collection. The radiological appearance could be a complex lesion and on MRI could show a peripheral enhancement that sometimes can show a rude aspect. Sequelae include increased risk of infection, especially if there is axillary dissection. Radiation-induced cancer is unusual. The most common are lung cancer, breast cancer, leukemia, and radiation-induced sarcoma. There is cumulative incidence of radiation-induced sarcoma, from 0.07% 5 years after the radiotherapy to 0.48% 15 years after radiotherapy, with a latency period of 5–7 years after radiotherapy. The clinical presentation often mimics benign pathology (usually skin thickening that can be confused with normal posttreatment changes), which explains the delay in diagnosis and thus advanced staging with worse prognosis.

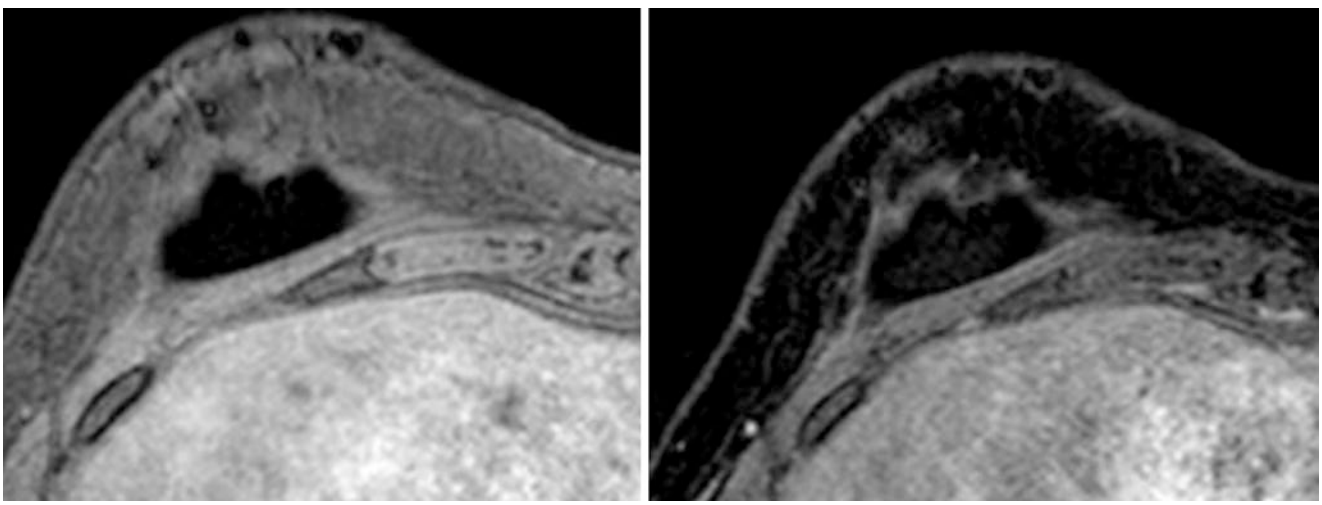

Fig. 21.13 Black silicone (on the *left*) and thrive postcontrast (on the *right*) sequences in a patient with previous history of left mastectomy and right symmetrization with mastopexy surgery unnoticed on other sequences. Postoperative changes on skin are seen

21.3 Imaging Findings After Surgery with Heterologous Material

Heterologous reconstruction is the most common type of reconstruction of an operated breast. The surgeon makes an incision in the skin usually including the NAC and removes the breast parenchyma through it. A pocket covered by a piece of skin is created where an implant will be placed (usually under the pectoralis major to prevent movement). The procedure may involve biological mesh and acellular dermal matrix to assist with the implant. It is advantageous over autologous reconstruction in several respects, including lower morbidity rates because there is no donor site, higher simplicity as it requires the shortest surgical time and recovery time, similar aesthetic presentation in color, texture, and sensitivity of the reconstruction to the adjacent tissue, and fewer scars. However, disadvantages include a very large and/or hypertrophic contralateral breast (usually ptotic) and a lack of a natural-looking breast causing patient refusal of the procedure. It is also contraindicated when there is poor skin quality due to RT or previous scars and insufficiency of the skin or pectoralis major to cover the implant.

More recently, free injection methods of different types of substances into the mammary gland have been also developed with a similar aim to that of implants, which is to increase breast size and to rebuild after surgery. They are especially useful in cases of partial defects. However, they remain controversial due to safety concerns including the possibility of migration of the injected substance to other parts of the body, their ability to promote the development of breast cancer, and the difficulty in assessing normal breast parenchyma after the procedure due to radiological findings and complications. Heterologous substances used in free injection techniques include free silicone, paraffin (no longer used), or hyaluronic acid (banned in some countries). These substances remain located in the mammary gland or in or under the pectoral muscle.

21.3.1 Implants

Clinical examinations are limited in their ability to evaluate implants and have low sensitivity for detecting ruptures. Thus, imaging techniques such as mammography, ultrasound, and MRI are clinically important.

21.3.1.1 Radiological Tests

The use of mammography is limited to evaluating the integrity of implants: extracapsular rupture, evident deformities, and capsular contractures, sometimes with calcifications. Silicone gel and saline implants will show a hyperdense appearance on mammogram (Fig. 21.14). The filling valve may be seen in the case of saline implants (Fig. 21.15). When there is a double lumen implant, both chambers can be distinguished; the inner chamber (saline) appears less dense than the outer chamber (silicone) (Fig. 21.16). Breast cancer screening in patients with implants will be affected since implants decrease the parenchymal visibility by 30–50%. Therefore, a full mammographic exploration should include:

- Oblique projection (both breasts).
- Cranio-caudal projection (both breasts).
- Eklund-modified compression technique, which is used in addition to the routine two-projection mammogram. It consists of posterosuperior displacement of the implants simultaneous to an anterior traction of the breast, pushing the implants toward the chest wall up to flatten. It provides significant improvement in image quality and displays a greater amount of breast tissue (Figs. 21.14 and 21.15).

Both silicone and saline implants have an anechoic appearance on ultrasound. Therefore, it is often not possible to distinguish sonographically between both if the patient's clinical history is not available (Fig. 21.17). Sometimes, however, it is possible to make a distinction if the filling valve of the saline implant is apparent (Fig. 21.18). The presence of a reverberation artifact and some subcapsular folds are normal findings. The main advantages of ultrasound are that it is a safe and noninvasive test, allows the integrity of the implant to be analyzed, allows the detection of extracapsular rupture (highly specific) and intracapsular rupture (where ultrasound's usefulness is more limited), and is the imaging technique of choice in guiding fine needle aspiration (FNA) or core needle biopsy to clarify suspicious lesions. Ultrasound provides a direct view of the breast in real time without risk of damage to the implant. The main limitations of ultrasound are that it is less capable for assessing the posterior aspect of the implant, it is highly dependent on the operator, and there are pitfalls when there is a lack of clinical history of the patient. For example, a hyperechoic line corresponding to the elastomer of the inner chamber in a double-lumen implant may be misinterpreted as a subcapsular line, leading to an incorrect diagnosis of an intracapsular rupture (Fig. 21.23a). Additionally, especially in very old implants with capsular contracture and with clinically suspected rupture, it is very common to find calcifications in the implant as well as ill-defined margins of the external capsule in the implant which may be misinterpreted as extracapsular rupture when there is no previous mammogram or CT for comparison.

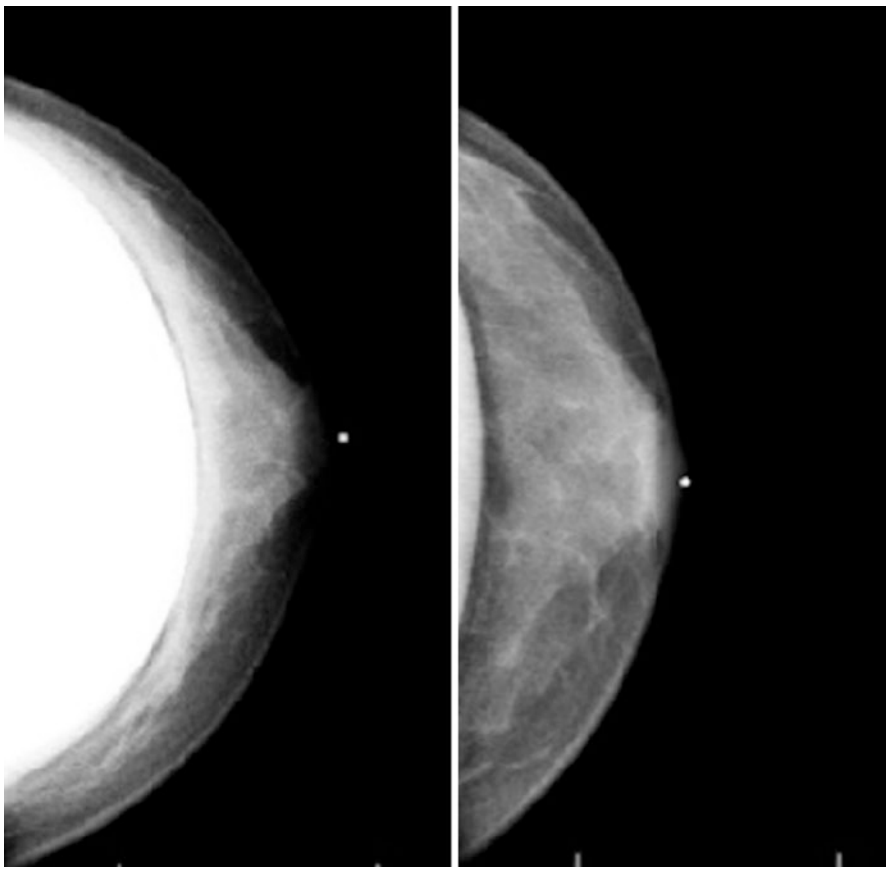

Fig. 21.14 Left breast mammography with CC projections in a patient with silicone gel implant. The silicone gel implant shows a hyperdense appearance on mammogram. The breast parenchyma is partially seen due to the implant (on the *left*), but with Eklund projection (on the *right*), the silicone gel implant can be displaced posteriorly (while the technician pushes the parenchyma to the anterior) to show most of it. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

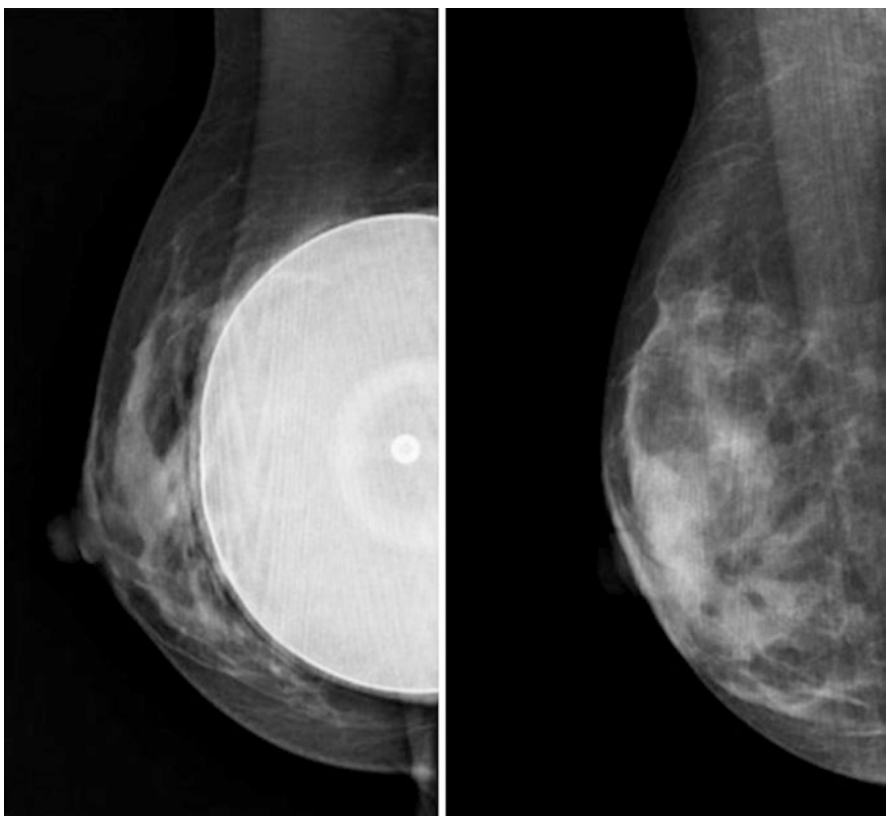

Fig. 21.15 Right breast mammogram with oblique projection in a patient with saline solution implant. The saline solution implant has a hyperdense appearance on mammogram, and the filling valve is seen at the center of the implant. The breast parenchyma is partially seen due to the implant (on the *left*). With Eklund projection (on the *right*), the saline solution implant is displaced to the posterior (while the technician pushes the parenchyma to the anterior) to show most of the parenchyma. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

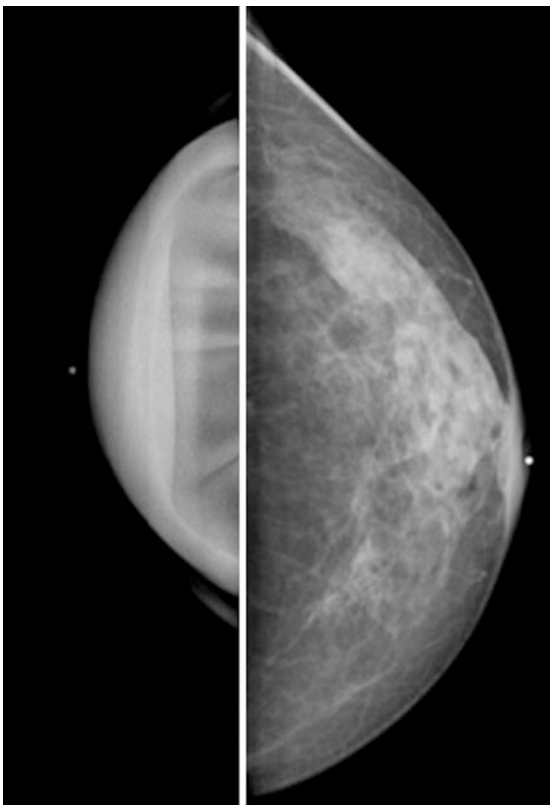

Fig. 21.16 Right breast mastectomy with a double-lumen expander. On mammography both chambers are identified. The inner chamber presents less density due to the saline solution

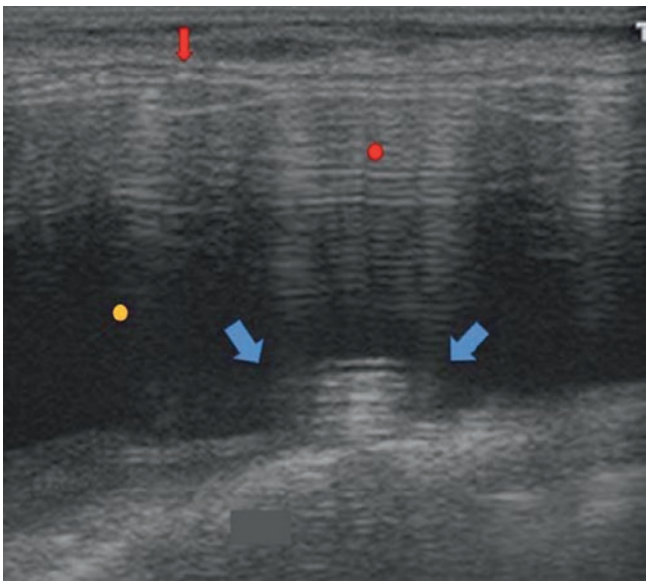

Fig. 21.18 Ultrasound appearance of a saline solution implant: Oval and anechoic mass identical to the silicone implant in Fig. 21.17 with a double hyperechoic line corresponding to the elastomer (*red arrow*) and reverberation artifact (*red dot*). The saline solution inside the implant presents an anechoic appearance (*yellow dot*). The only way to differentiate this implant from the silicone implant is to appreciate the filling valve in its posterior aspect (*blue arrows*). Sometimes it is not well seen with ultrasound because of the depth of the posterior margin of the implant. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica.

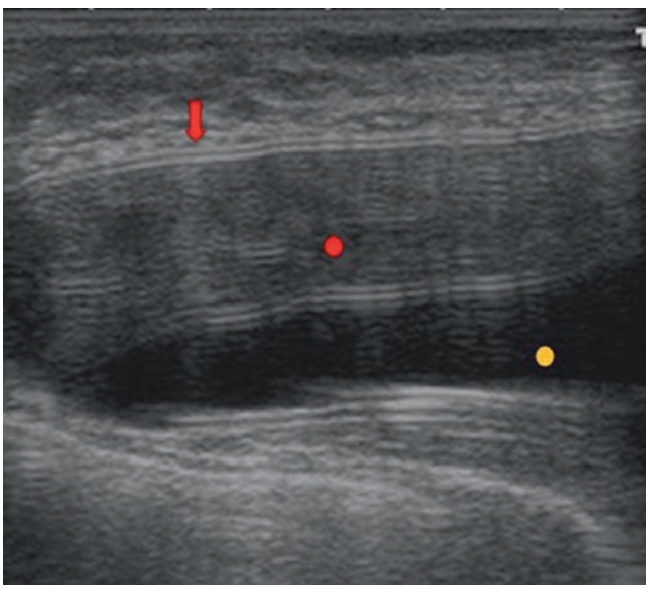

Fig. 21.17 Ultrasound appearance of a silicone gel implant: Oval and anechoic mass where the elastomer appears as double hyperechoic line (*red arrow*) with reverberation artifact below (*red dot*), and the silicone gel shows an anechoic appearance (*yellow dot*). There is no filling valve. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica.

Breast MRI is the most sensitive and reliable imaging technique available to assess implants and their associated complications. To obtain high-quality images, the use of high-field MRI machines with a breast-specific coil is required. MRI is performed when the patient is in a prone position to avoid respiratory movements that can affect the quality of the images. To examine silicone gel implants (Fig. 21.19), the protocol should include specific sequences for silicone, for example, white and black silicone sequences. The white silicone sequence is especially useful as the image will show only the white silicone, either intra- or extracapsular. Sagittal sequences should also be included so sutile intracapsular ruptures can be appreciated and the inferior edge of the implant can be better analyzed. One option is to perform the T2 sagittal sequence without fat suppression or to perform fine-slice imaging in the axial plane and sagittal reconstruction posteriorly. The use of an intravenous contrast agent is not necessary if MRI is only intended to evaluate the implant. However, it is useful if the patient has a history of breast cancer or a high-risk lesion. Silicone implants will have the following

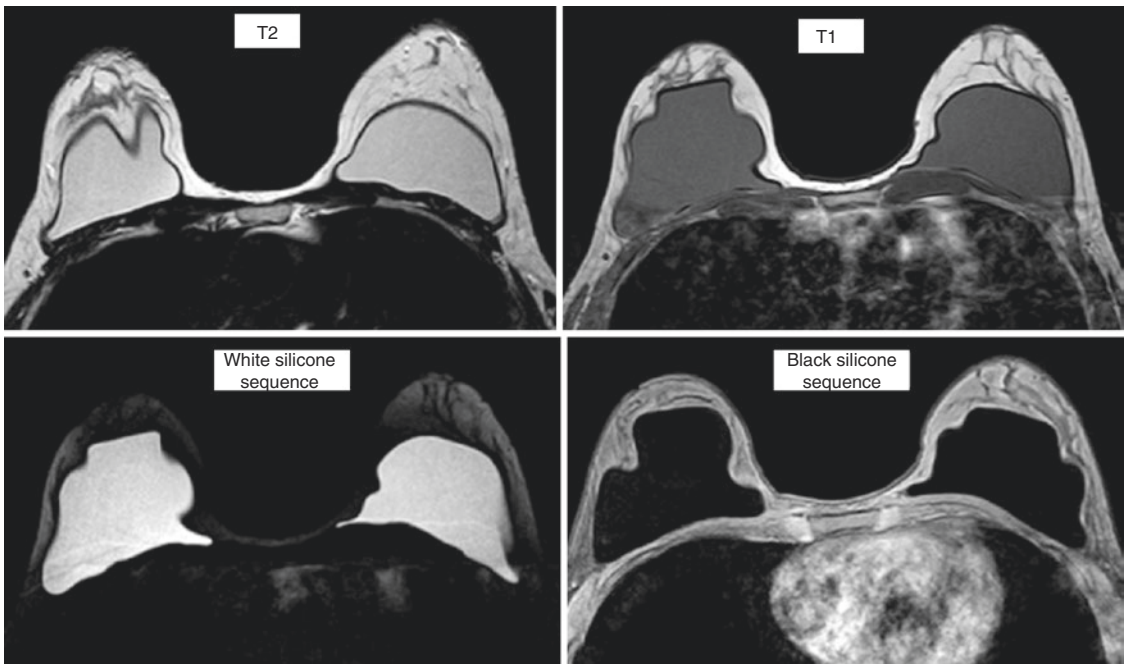

Fig. 21.19 Patient with silicone implants. Normal appearance of the silicone in different sequences

appearance on the different MRI sequences (Figs. 21.19 and 21.23):

- T1 sequence: Hypointensity.
- T2 sequence: Hyperintensity but not as intense as in a white silicone sequence or compared with a saline implant.
- STIR/SPAIR sequence: Hypointensity although sometimes this can vary depending on the composition of the implant.
- White silicone sequence: Marked hyperintensity, which contrasts with the rest of the breast.
- Black silicone sequence: Marked hypointensity.

Specific sequences for silicone make no sense if the implant is a single-lumen saline implant, since the implant will look exactly like liquid in all sequences (Fig. 21.20).

MRI is not useful to assess expanders with an anterior valve, since this has a metallic component (that can be seen on X-ray and on CT) (Fig. 21.21), and so an artifact is produced on that hemithorax (Fig. 21.22). However, it is possible to perform the MRI to assess the contralateral breast; although the valve can move a little because of the magnetic field, the surgeon, posteriorly, is able to locate the filling valve over a magnet. The only obstacle to MRI is if the patient notes a warm or burning sensation on the skin.

In cases of a bicameral implant, the filling valve is made of titanium and usually located adjacent to the chest wall, lateral to the implant and connected with it through a connector. Then, the patient may be subjected to breast MRI to assess both the breast with implant and the contralateral breast because there is no artifact on that hemithorax. Besides, there is no risk of implant movement or warmth on the skin. It is advisable to visualize all the connector routes and the valve to detect possible complications and ruptures. For that purpose, black silicone and thrive postcontrast sequences are useful (Fig. 21.23).

The main advantages of MRI are that it is a noninvasive technique, is useful in breast cancer screening as it achieves a better assessment of parenchymal lesions with the administration of intravenous contrast, and allows the assessment of the implant in three projections and the evaluation of intracapsular and extracapsular ruptures. It may show migration of silicone to lymph nodes (axillary and internal mammary) as well as soft tissue when there is an extracapsular rupture.

However, it is not free of disadvantages, such as cost, availability, motion and breathing artifacts, and false-positive cases.

21.3.1.2 Radiological Findings

Some findings are considered as normal on MRI, for example, a small amount of periprosthestic fluid; rippling, subcapsular folds (these can be differentiated from an intracapsular rupture because most times they have fluid on one side); and fibrous bands.

Complications include intracapsular rupture, extracapsular rupture, migration of the silicone gel to lymph nodes and

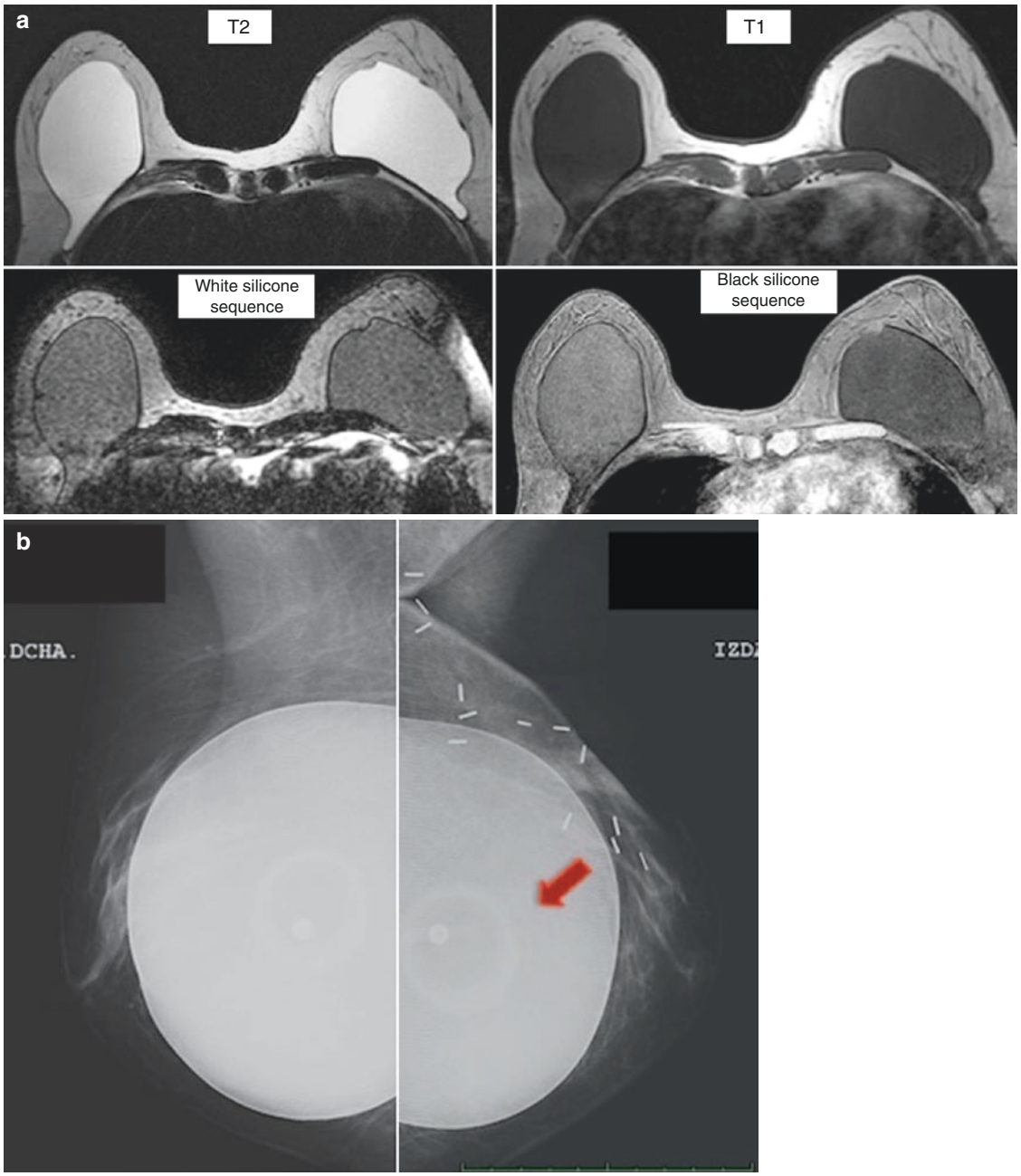

Fig. 21.20 Patient with breast-conserving surgery in UOQ of the left breast and reconstruction with saline solution implants. (**a**) MRI was performed without the presence of a radiologist, and the type of implants was unknown, so white silicone and black silicone sequences were made. The saline solution implants show identical signal intensity of the liquid which was hypointense on T1 and hyperintense on T2 (higher hyperintensity than silicone). On black and white silicone sequences, implants show similar signal intensity, slightly hypointense. This presentation may present a pitfall, suggesting a technical failure of the machine that is not able to suppress appropriately. (**b**) In this case and following a review of previous explorations, the patient had a mammogram where the filling valves could be seen in the posterior aspect of the implant (*red arrow*) supporting that these were saline implants

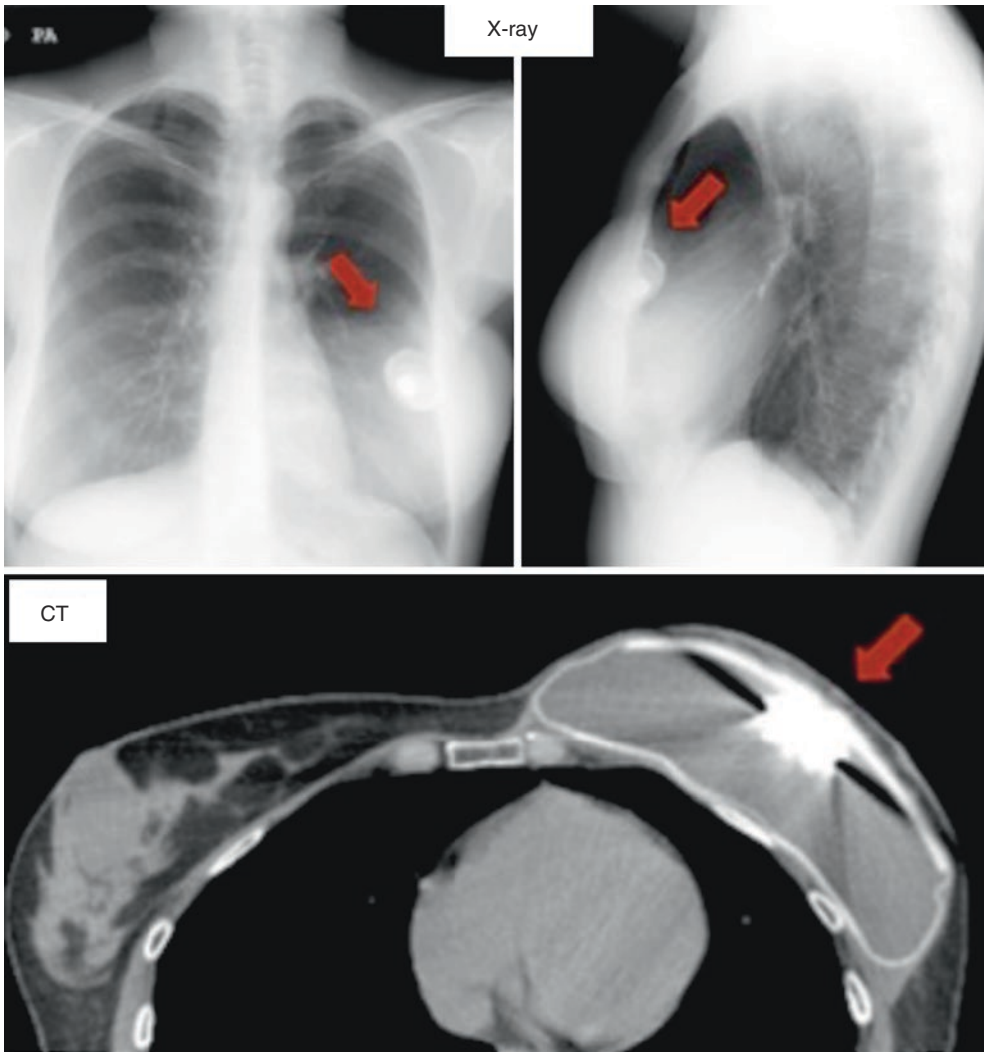

Fig. 21.21 Patient with left mastectomy and heterologous reconstruction with a unicameral saline solution expander with filling valve. The metallic component allows the expander to be seen on radiographs and on CT. The filling valve and the metallic artifact can be noticed (*red arrow*)

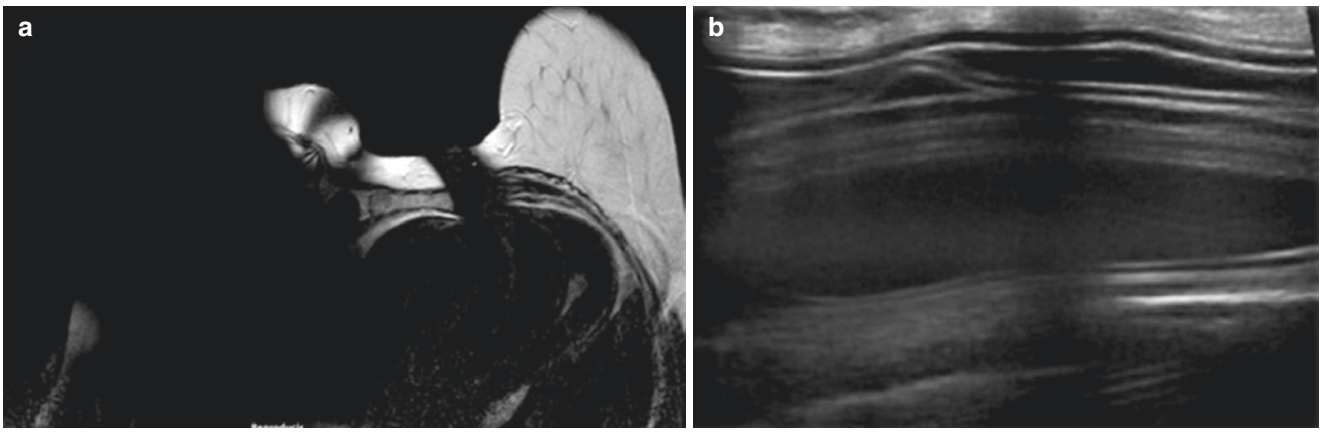

Fig. 21.22 Patient with personal history of right mastectomy and temporary reconstruction with an expander with a metallic valve. Breast MRI was performed due to surgeon request in the case of suspected rupture. There was no contraindication for the MRI except that it was not useful to evaluate either the operated breast or the expander, being able to only to assess the contralateral breast. (**a**) T2 sequence axial plane: The metallic component of the filling valve produces a magnetic artifact that prevents evaluation of the expander. However, the contralateral breast is clearly seen. (**b**) An ultrasound confirms collapse of the expander

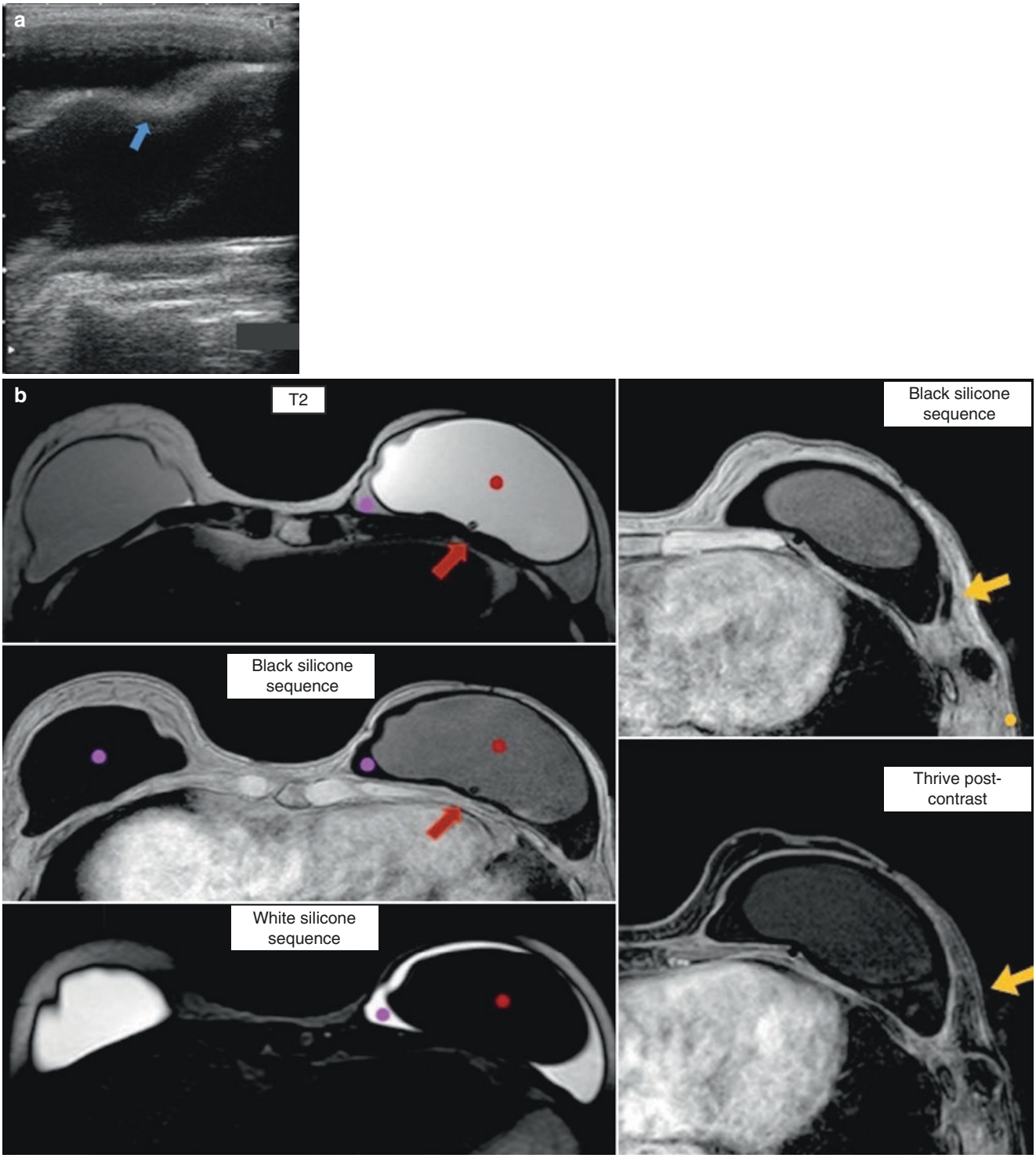

Fig. 21.23 Patient with bilateral mastectomy and left reconstruction with bicameral expander and right reconstruction with silicone implant. (**a**) Ultrasound: Two chambers separated by a hyperechoic line can be seen. It is a very common pitfall to misunderstand that line as the subcapsular line seen in intracapsular ruptures. (**b**) MRI with different sequences: Two chambers with different signal intensities can be seen. *Red dot*: internal chamber of saline solution. *Violet dot*: external chamber of silicone on the left breast and silicone implant on right breast. *Red arrow*: connector going from the posterior part of the expander through the lateral margin of the implant to the chest wall where the filling valve is located. Black silicone and thrive postcontrast sequences were very suitable to assess the connector in its path out of the implant (*yellow arrow*) and the filling valve (*yellow dot*). The connector should be assessed over its entire route to ensure that there are no ruptures

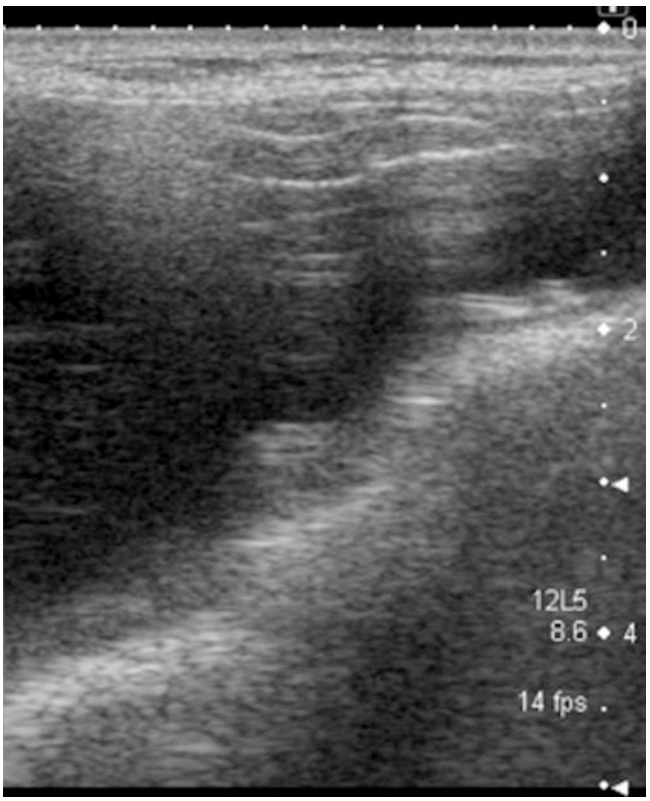

Fig. 21.24 Ultrasound showing an intracapsular rupture: stepladder sign. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

soft tissues, capsular contracture (where the diagnosis is usually clinical), and displacement/herniation (where the diagnosis is usually clinical) (Fig. 21.30).

1. Radiological signs of intracapsular rupture:

It is difficult to detect intracapsular rupture on clinical exam or mammography. This complication is better seen on ultrasound or MRI, with the latter as the best option:

- On ultrasound:
  - Overall sensitivity and specificity rates range between 59–85% and 55–79%, respectively.
  - "Stepladder sign": Hyperechoic lines parallel to the elastomer resembling a ladder or railway (Figs. 21.24 and 21.28a).
  - Subcapsular irregular and discontinuous hyperechoic lines inside the implant (Figs. 21.25 and 21.29).
  - Hyperechoic content inside the implant (Figs. 21.25 and 21.29).
- On MRI:
  - Unicameral implant:

    Without collapse: "*Keyhole*," "*teardrop*," "*inverted loop*," or "*noose*" signs (Figs. 21.26 and 21.27). An implant without collapse appears as silicone on both sides of a radial fold. These signs indicate focal extravasation of silicone, which is confined to the fibrous capsule without extending cir-

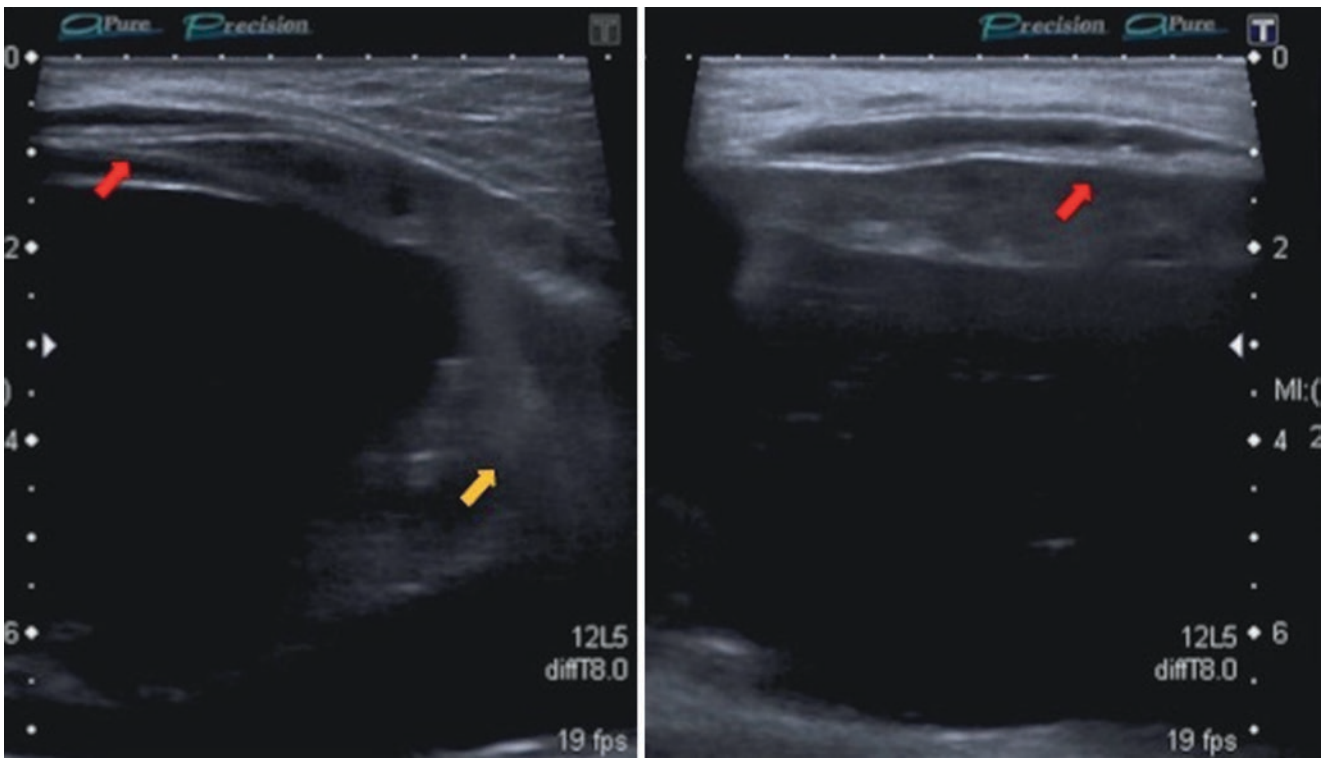

Fig. 21.25 Ultrasound showing an intracapsular rupture: Irregular subcapsular line under the shell (*red arrow*) and hyperechoic content and lines inside the implant (*yellow arrow*)

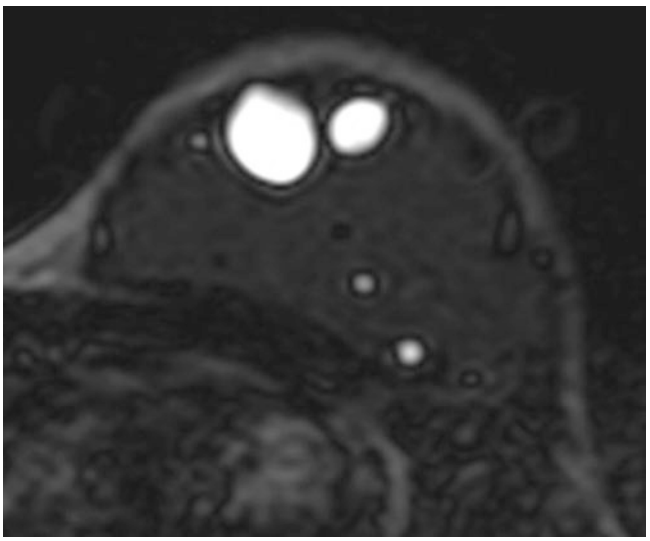

Fig. 21.26 Breast MRI with black silicone sequence: teardrop sign. The silicone appears completely black, and inside there are some white images due to water

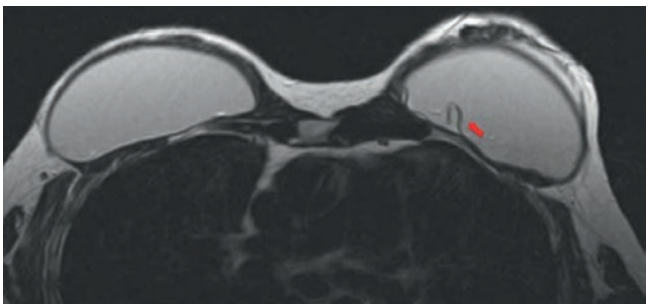

Fig. 21.27 Breast MRI with T2 sequence: inverted loop sign. There is a loop originating from the shell that goes inside the implant, with silicone on both sides

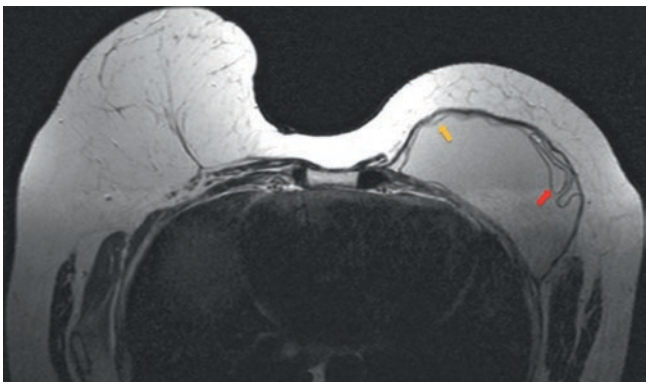

Fig. 21.28 Breast MRI with T2 sequence without fat suppression showing an intracapsular rupture: Linguini sign (*red arrow*) and a subcapsular line (*yellow arrow*)

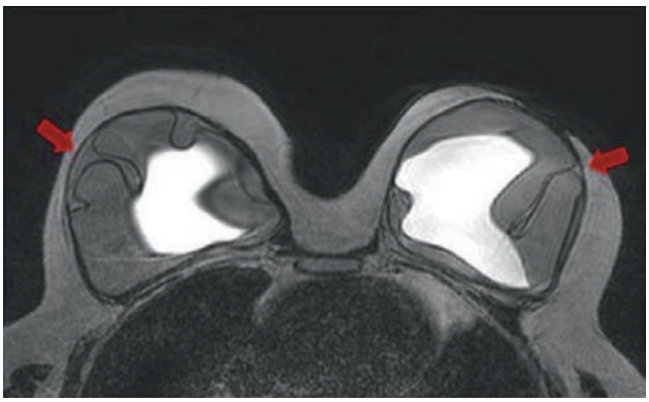

Fig. 21.29 Patient with bilateral mastectomy with heterologous reconstruction involving double-lumen expanders with definitive intention. Breast MRI (T2 sequence, axial plane): Both chambers can be seen in both breasts. The inner chamber is white because it consists of saline solution, and the outer one is gray because it consists of silicone gel. A subcapsular line (*red arrow*) is seen beside both outer chambers with silicone signal on both sides of the line indicating bilateral intracapsular rupture

cumferentially due to the presence of adhesions at their ends. However, none of these signs is a reliable sign of rupture on its own. Other imaging features, assessed in combination, may better suggest intracapsular rupture.

With minimal collapse: "*Subcapsular line*" (Fig. 21.28). There is presence of a parallel and hypointense line to the fibrous capsule with silicone on both sides. It represents an evolution of the previous stage, but in this case the base of the invagination of the elastomer is greater than in cases with no collapse.

With partial or total collapse: "*Linguini*" or "*wavy line*" signs (Fig. 21.28) due to a free-floating shell within the implant. Internal and hypointense curved lines are seen without a perpendicular orientation to the shell. It represents rupture of the elastomer and the presence of silicone between it and the fibrous capsule. It is the most reliable sign of an intracapsular rupture with a sensitivity and specificity of 96 and 76%, respectively.

– Bicameral implant or expander: If the rupture is in the outer chamber, findings are similar to that of an intracapsular rupture in a single-lumen implant (Fig. 21.29). If the rupture is in the inner chamber, a "*salad oil*" sign or "*mixed signal intensities*" between both chambers may be seen. Subcapsular lines inside the saline chamber may also be seen (Fig. 21.30). As the saline chamber is ruptured, a decrease in the volume of the implant is also noticed.

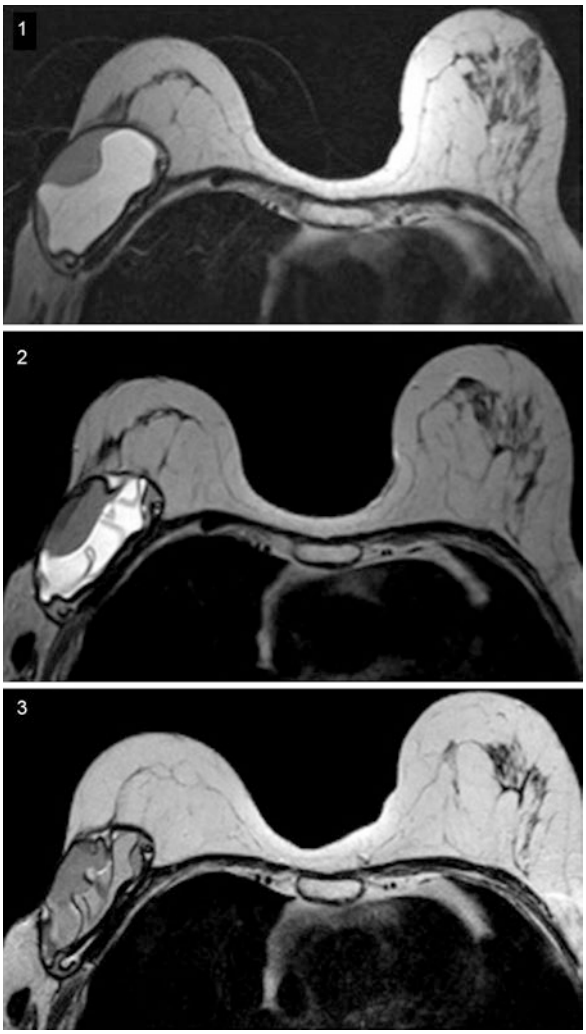

Fig. 21.30 Patient with conserving surgery in the right breast with reconstruction using a double-lumen expander. Follow-up breast MRI was performed every year. T2 sequences are shown each year. In year 1, the double-lumen implant is seen with both intact chambers. However, herniation signs and capsular contracture are noted. In year 2, the inner chamber (with saline solution) shows subcapsular lines with the same signal on both sides of the line indicating an intracapsular rupture. However, the patient did not replace the implant. In year 3, the subcapsular lines inside the inner chamber persisted, and additionally a mixed signal intensity is seen not showing the typical white appearance of fluid signal

2. Radiological signs of extracapsular rupture:

Extracapsular rupture is evident as the silicone, when separated from the implant, extends beyond the implant capsule into the breast or axilla. The silicone shows up as free silicone in the parenchyma, as granuloma (siliconoma) when the body encapsulates the silicone that may manifest as a lump or tumor, or as silicone migration in lymph nodes and soft tissues.

The presence of silicone outside the fibrous capsule without evidence of fibrous capsule or shell rupture can occur in two circumstances:

- Previous implant rupture and posterior replacement where there are remains of previous extracapsular silicone in the breast (Fig. 21.31).
- Leakage of silicone due to permeability of the shell and/or the fibrous capsule. The lymphatic vessels pick up this leakage and carry it to the lymph nodes. The lymph nodes can present a snowstorm sign or simply show enlargement without a fat center because of reactive lymphadenitis (Fig. 21.32). Differential diagnosis should be done with extracapsular rupture with silicone migration to the lymph nodes and with lymph node involvement because of tumor.

Radiological signs of extracapsular rupture are:

- On mammography:
  - Oval and well-defined hyperdense masses outside the implant.
  - Change of the contour of the implant that may be detected on clinical exam or mammography.
  - Enlarged and hyperdense axillary lymph nodes due to silicone migration.

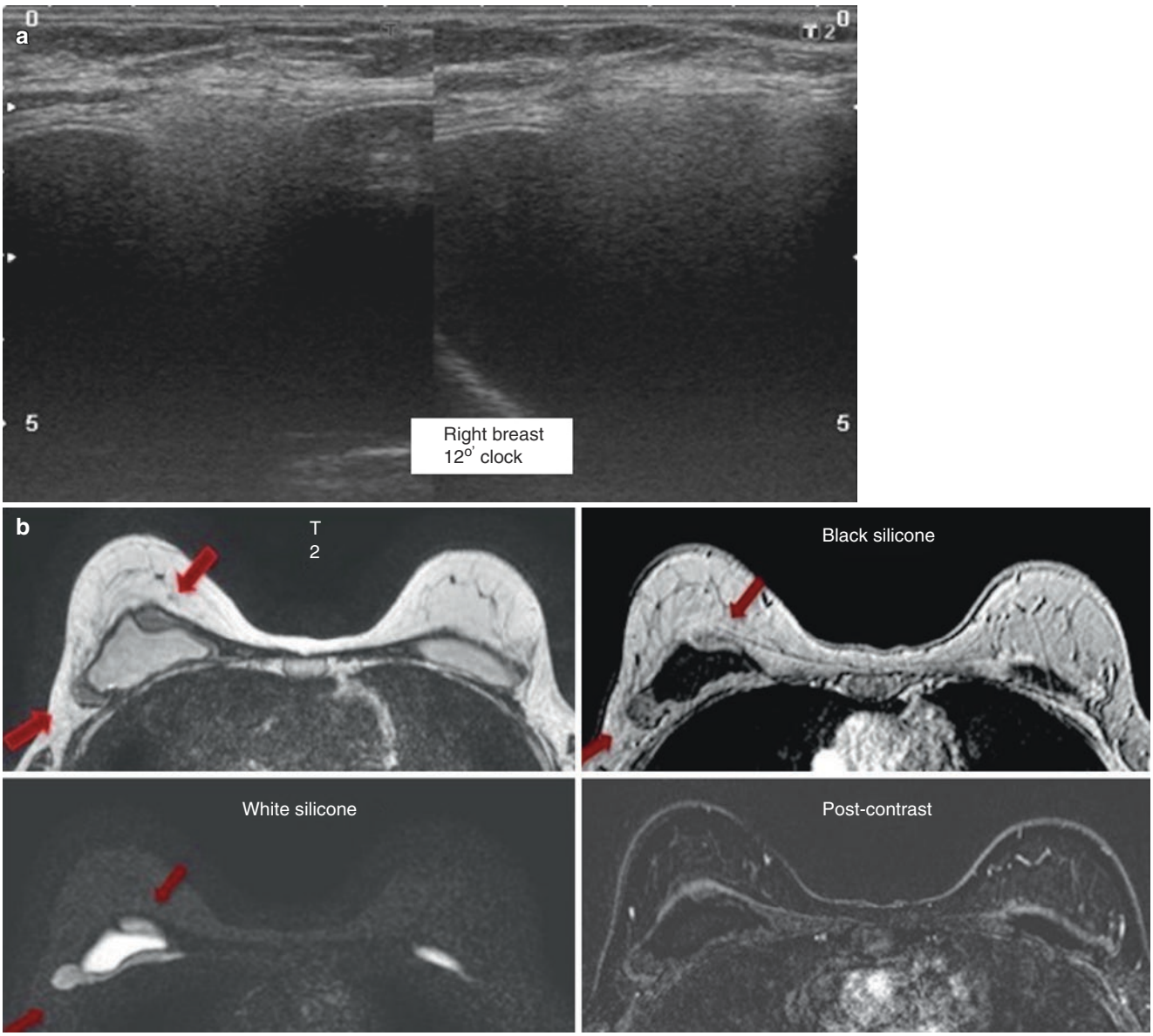

Fig. 21.31 Patient with personal history of extracapsular rupture with silicone extravasation. The surgeon referred the patient for replacement and cleaning. (**a**) Ultrasound was performed in a screening and a snowstorm sign was around some areas of the implant that prevented assessment of the capsule, suggesting extracapsular rupture. The patient wanted a definitive diagnosis before a new replacement surgery. (**b**) MRI was performed. An extracapsular material around the implant was seen, with signal intensity that did not correspond to free fluid but was similar to silicone (hyperintense) in silicone sequences (*red arrow*) and did not enhance after IVC. This could suggest a new extracapsular rupture; however, this signal intensity was not identical to intracapsular silicone on other sequences, and no evidence of discontinuity or rupture of the shell or fibrous capsule was seen. Therefore, these findings suggested remainders of the previous extracapsular rupture more than a new extracapsular rupture. That fact was confirmed with core needle biopsy with granulomatous reaction to foreign body result

- On ultrasound:
  - "*Snowstorm*" sign (Figs. 21.31 and 21.33): Highly specific. Extracapsular silicone appears as a hyperechoic image that prevents transmission of the ultrasound beam and the image resembles falling snowflakes.
  - Hypoechoic nodular lesions that are difficult to distinguish from solid nodules, usually due to a granulomatous reaction to a foreign body (silicone).
- On breast MRI:
  - Breast MRI is the most sensitive imaging technique for detecting small foci of migration.
  - In recent ruptures, free silicone shows a similar signal to the signal of the interior of the implant in all sequences, and there is no enhancement. However, over time, as granulation tissue builds up, the signal intensity changes and may present some enhancement after IVC administration.

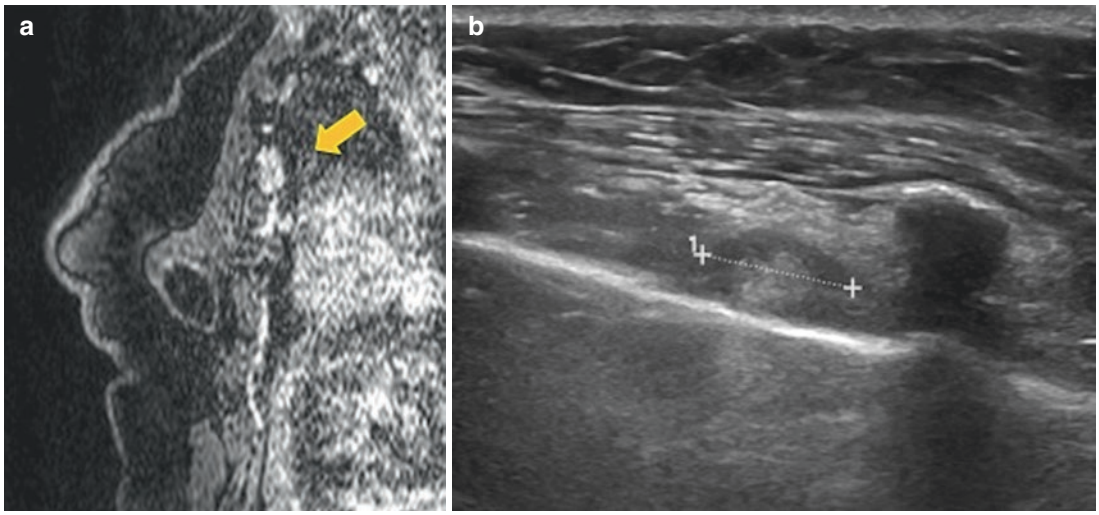

Fig. 21.32 Patient with bilateral mastectomy and heterologous reconstruction with an intracapsular rupture of the right implant. MRI showed an enlarged mammary internal lymph node that enhanced after IVC administration. (**a**) Dynamic sequence sagittal reconstruction shows an enlarged internal mammary lymph node without fat center (*yellow arrow*). Differential diagnosis should be done for an extracapsular rupture with silicone migration to lymph nodes, implant leakage with reactive lymphadenitis, or lymph node involvement due tumor as the patient had personal history of breast cancer. However, an extracapsular rupture was not seen and the external capsule was preserved. (**b**) Fine needle aspiration biopsy under ultrasound guidance was performed of that lymph node and the result was reactive lymphadenitis

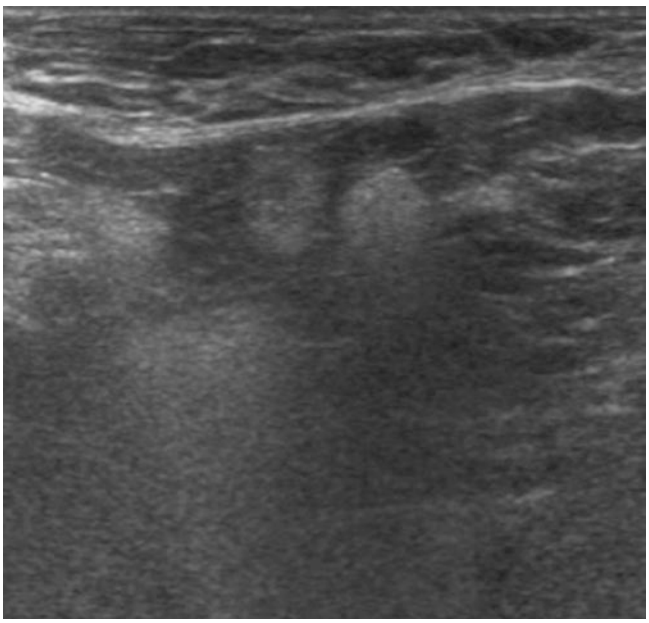

Fig. 21.33 Ultrasound showing hyperechoic lymph nodes with the snowstorm sign indicating extracapsular rupture and silicone migration to axilla. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica.

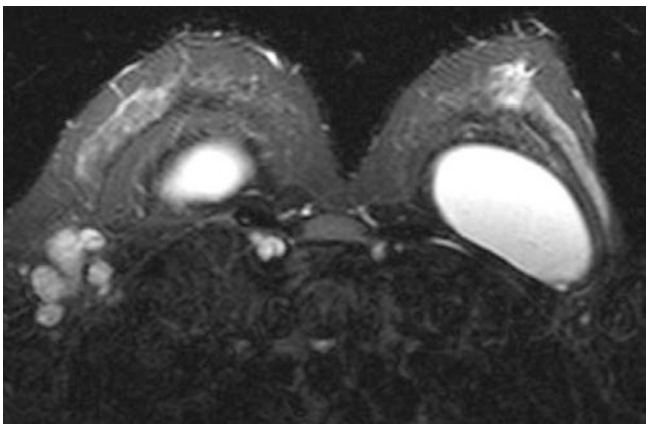

Fig. 21.34 Breast MRI, white silicone sequence, showing extracapsular silicone in the axilla and internal mammary lymph nodes. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica.

– Extracapsular silicone can be seen in the parenchyma, lymph nodes, or soft tissues (Fig. 21.34).

21.3.1.3 Follow-Up Protocol

1. Patients without a personal history of breast cancer:

Ruptures are frequently asymptomatic, and a clinical examination alone is not enough for diagnostic purposes. It is necessary to establish a diagnostic algorithm based on imaging in these patients.

Many studies show MRI as the technique of choice for evaluating the integrity of implants with a sensitivity of 72–94% and specificity of 85–100% and its superiority in detecting intracapsular rupture. This superiority of MRI, as well as the fact that the risk of rupture increases with implant longevity, has led the US FDA to recommend screening with MRI every 2 years from 3 years since the implant placement. However, due to its high cost and low availability, it is not routinely used as a screening method.

We recommend performing mammography as is performed for other patients without implants, according to age

and personal and family history but with the addition of Eklund projections and/or ultrasound.

This protocol could be:

- Mammography + Eklund projections ± Ultrasound
- Breast MRI when there are doubtful findings on mammography and ultrasound or when there are normal findings on mammography and ultrasound, but there is still a clinical suspicion of rupture.

2. Patients with a personal history of breast cancer:

Physical examination is more sensitive in operated patients. This is because over time the implant causes atrophy of the parenchyma and the implant shifts it to the skin (Fig. 21.35).

In addition, the sensitivity of mammography decreases over time. As such, we recommend that screening of these patients should include Eklund projections ± breast ultrasound. Breast MRI has the greatest sensitivity (Fig. 21.35), but it should be only used in doubtful cases of rupture. However, if the patient is younger than 50 years of age and has personal history of breast cancer and reconstructive surgery with implants, some protocols recommend an annual breast MRI as a screening method with the mammography and ultrasound.

Different options:

For symptomatic patients with suspected ruptures, ultrasound is the imaging technique of choice at the time of symptoms.

For asymptomatic patients, it is best to perform regular screening according to age but with the addition of Eklund projections and possibly ultrasound.

For patients with a history of breast cancer, specifically, if they have breast-conserving surgery and reconstructive surgery with an implant, it is best to perform a bilateral mammography along with Eklund projections and ultrasound annually. In patients who have had mastectomy and then reconstructive surgery with an implant, an annual ultrasound is recommended; mammography is not performed except in some cases where a single mammography is performed postsurgically to check for the absence of residual breast parenchyma.

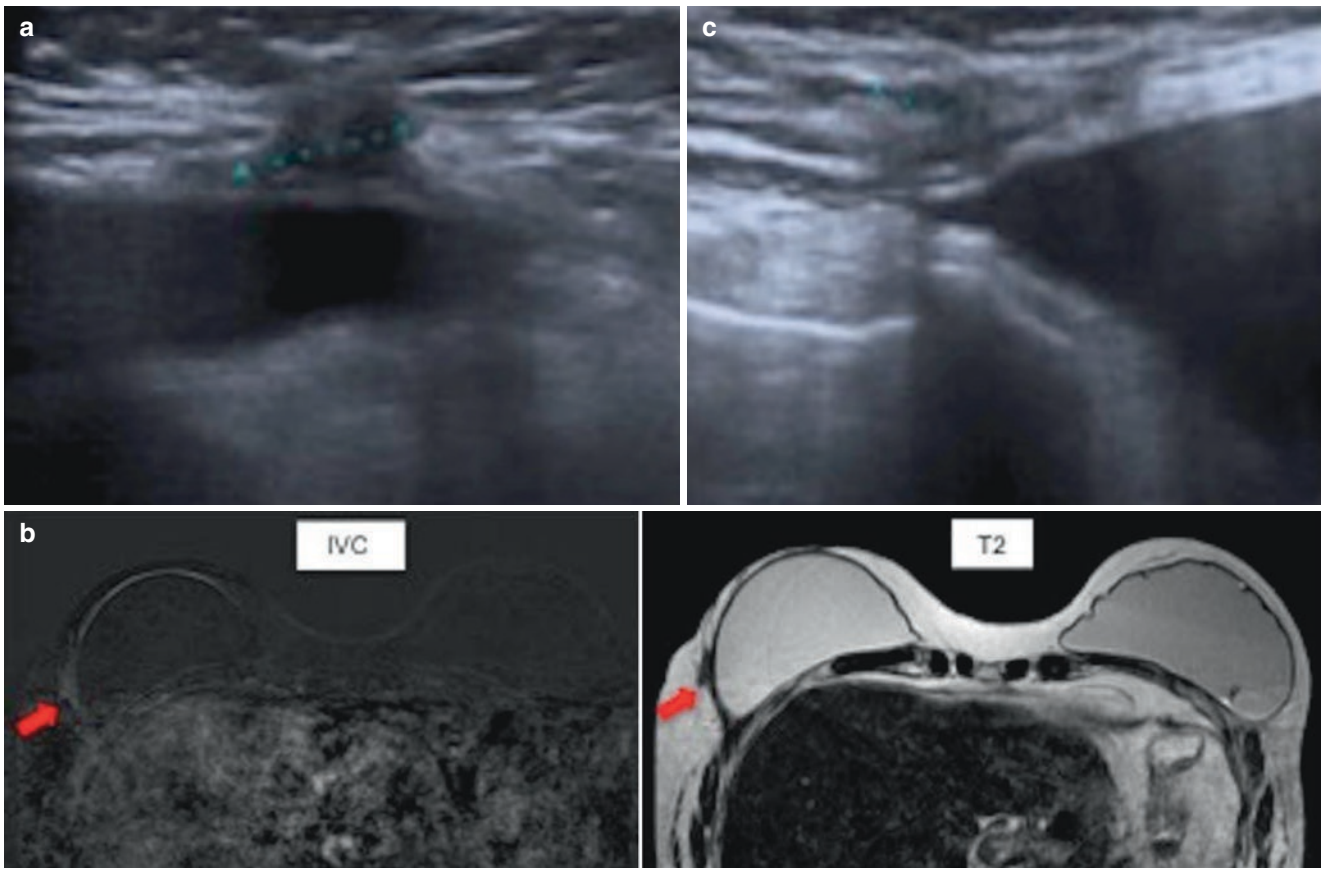

Fig. 21.35 Patient with a personal history of bilateral mastectomy. The patient noted a lump in the LOQ of right breast. (**a**) Ultrasound showing an ill-defined margin lesion in that location, adjacent to the implant, highly suggestive of malignancy. A breast MRI was performed due to difficulty in differentiating the suspicious finding from scar. (**b**) The lesion seen on ultrasound shows slight enhancement but irregular and ill-defined margins. (**c**) In a superior slice, another lesion is observed in the right UOQ with similar characteristics as the main lesion. (**d**) Second look ultrasound shows another ill-defined lesion in the UOQ, corresponding to the lesion observed on MRI. Both lesions were invasive ductal carcinoma confirming a multicentric recurrence after a bilateral mastectomy

Breast MRI is performed only in cases where conventional tests (mammography and ultrasound) are doubtful or inconclusive for rupture, when there are clinical suspicions of possible complications of the implants and as a screening method in patients with personal history of breast cancer younger than 50 years old.

21.3.2 Biological Mesh and Acellular Dermal Matrix

21.3.2.1 Radiological Tests and Findings

1. *Mammography*: Mammography is not usually performed in patients who have undergone mastectomy.
2. *Ultrasound*: Ultrasound is prone to pitfalls if it is unknown whether the patient has a biological mesh. Nodular or pseudo-nodular lesions are seen where the mesh is attached to the implant or the pectoral or serratus muscles (Fig. 21.36). They may present uncircumscribed margins and may lead to a core needle biopsy damaging the biological mesh.
3. *MRI* (Fig. 21.36) *and CT*: These tests help differentiate those images as they are usually bilateral and symmetrical (when the mastectomy and reconstruction are bilateral), show benign characteristics, and do not show enhancement after IVC administration.

21.3.3 Free Silicone Injection (Silicone Mastopathy)

21.3.3.1 Radiological Tests and Findings

Radiological features of free silicone are similar to that of an extracapsular rupture, although without an implant:

1. Mammography (Fig. 21.37):
   - Extremely dense masses ± rim calcification
   - Distortions
   - Breast asymmetries
   - Diffuse increase of parenchymal density complicating the display and assessment of suspicious lesions
   - Extremely dense lymph nodes
2. Ultrasound:
   - Nodular lesions with snowstorm sign
   - Nodular lesions with benign characteristics (oval and well defined, without shadowing)
   - Hypoechoic and/or heterogeneous nodules
   - Nodular lesions or areas with shadowing obscuring the posterior breast
3. MRI:
   - It is especially useful in clarifying clinical suspicions and/or uncertain diagnosis with ultrasound or mammography.

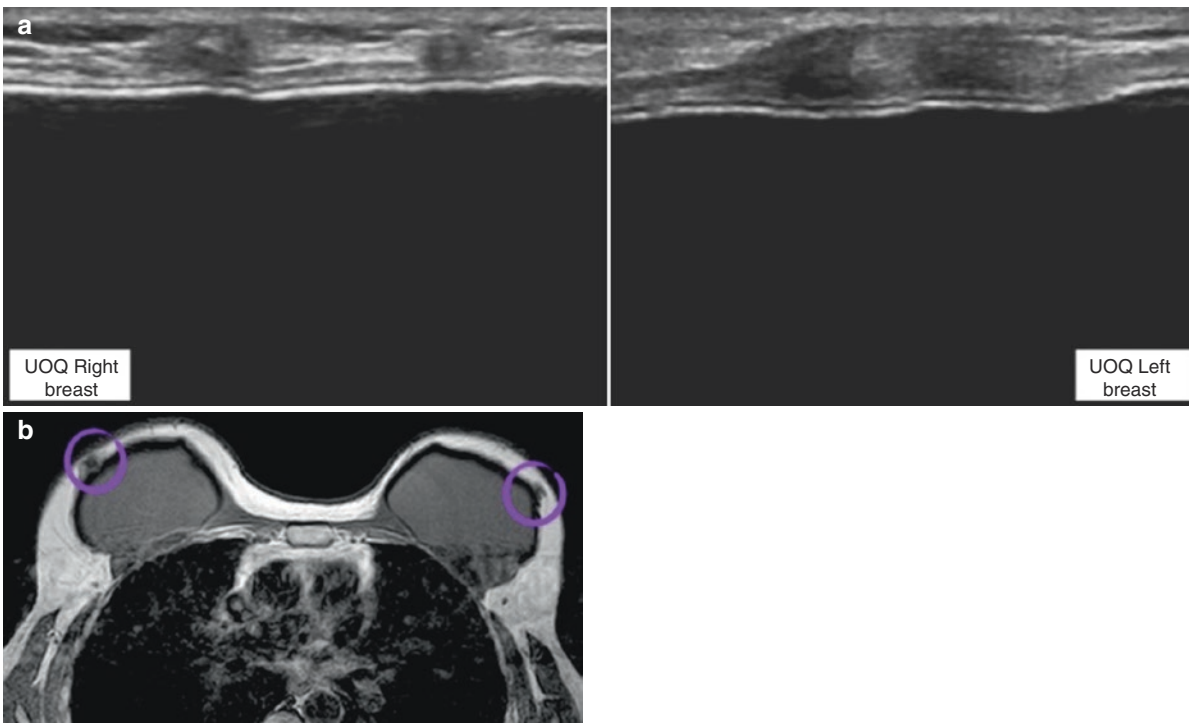

Fig. 21.36 Patient with bilateral mastectomy and heterologous reconstruction in a single surgical procedure with silicone implants and biological mesh. (**a**) First follow-up ultrasound shows nodular and solid appearance images, with ill-defined margins located in some locations. A core biopsy was recommended but as the patient had a recent bilateral mastectomy without complications and free margins, and an MRI was requested. (**b**) Breast MRI T1 sequence shows bilateral, symmetrical and hypointense nodular lesions in the junction areas of the mesh with the implant and the chest wall that had produced the sonographic pitfall

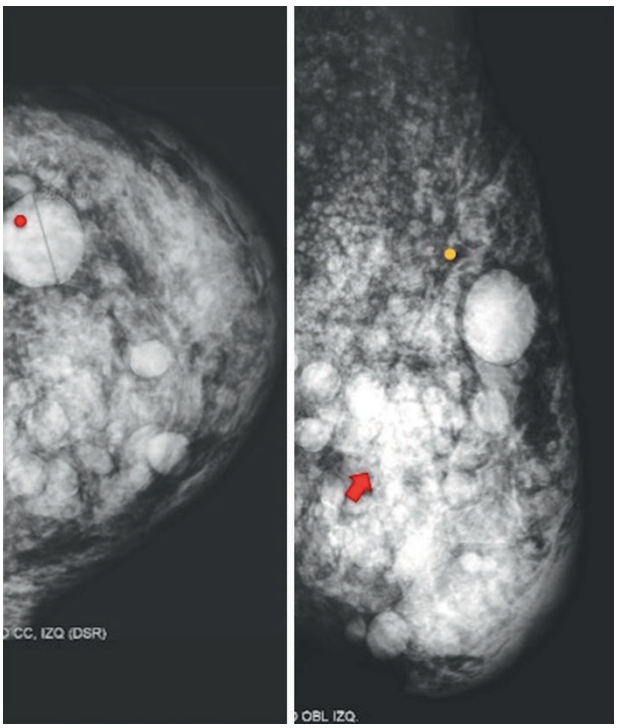

Fig. 21.37 Patient with free silicone injection in the thickness of the breast parenchyma. Right breast mammogram (oblique and craniocaudal views): There is a diffuse increase of breast density (*red arrow*), some distortion image (*yellow dot*), and some circumscribed and dense nodules (*red dot*). Some of these findings may be suspicious only with mammogram, preventing the dismissal of malignancy in the breast cancer screening. This image appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

- Nodules with benign characteristics, with the same signal as silicone, without enhancement after IVC.
- Nodules with peripheral enhancement after IVC administration due to granulomatous reaction. If there is some doubt, a cytohistological exploration should be done.

21.3.4 Hyaluronic Acid

21.3.4.1 Radiological Tests and Findings (Fig. 21.38)

On mammography and ultrasound, findings are similar to those for free silicone injection. On MRI, nodules are similar to fluid nodules except when there is a granulomatous reaction:

1. *Mammography*: Usually dense and well-defined masses
2. *Ultrasound*:
   - Solid appearance lesion.
   - Simple, complicated, and complex cystic lesion. Over time the lesions usually evolve to a more solid appearance.
   - Septate and interconnected collections.
3. *Breast MRI*:
   - Lesions with identical signal intensity as fluid on T1 and T2 sequences, without enhancement after IVC administration.
   - Lesions with peripheral enhancement when granulomatous reaction is produced.

In any case, sometimes hyaluronic acid can produce images mimicking solid lesions that may show enhancement. It can also hinder the display of remaining breast parenchyma and consequently the diagnosis of breast cancer if it occurs.

21.4 Imaging Findings After Surgery with Autologous Material

In breast surgery using autologous material, the surgeon uses autologous or endogenous material from the body of the patient.

Autologous reconstruction, where the surgeon uses flaps from the body of the patient, is the technique of choice of all reconstructions using autologous material. It is less commonly performed compared to heterologous reconstruction, but its use has dramatically increased over the years. Among other factors, the number of mastectomies has increased, and autologous reconstruction offers several advantages over heterologous reconstruction, for example, better aesthetic outcomes, more natural-looking reconstructions, durability, better results over a radiated skin, and moreover, the technique is not limited by the amount of available skin after surgery. However, it also has several disadvantages. It is surgically more complex than heterologous reconstructions using implants. It is associated with a higher rate of complications such as in donor site as in neobreast and higher morbidity.

Free injection techniques using different autologous substances have also been developed. Lipofilling is a free injection technique similar to the injection of free silicone and hyaluronic acid; however, the fat used in lipofilling is autologous, and the technique is completely accepted worldwide. Lipofilling has several advantages over other free injection techniques, for example, the autologous fat avoids the possibility of rejection and granulomas, increased risk of breast cancer has not been demonstrated, and the free fat will have a similar appearance than breast fat, so it should not interfere with radiological interpretation on imaging and consequently with cancer diagnosis. For all those reasons, it is commonly used as a reconstructive technique and aesthetic procedure. However, sometimes new palpable lumps may appear mimicking cancer on imaging, requiring breast MRI or biopsy.

NAC reconstruction is also possible using autologous material. It is the final step after breast reconstruction in

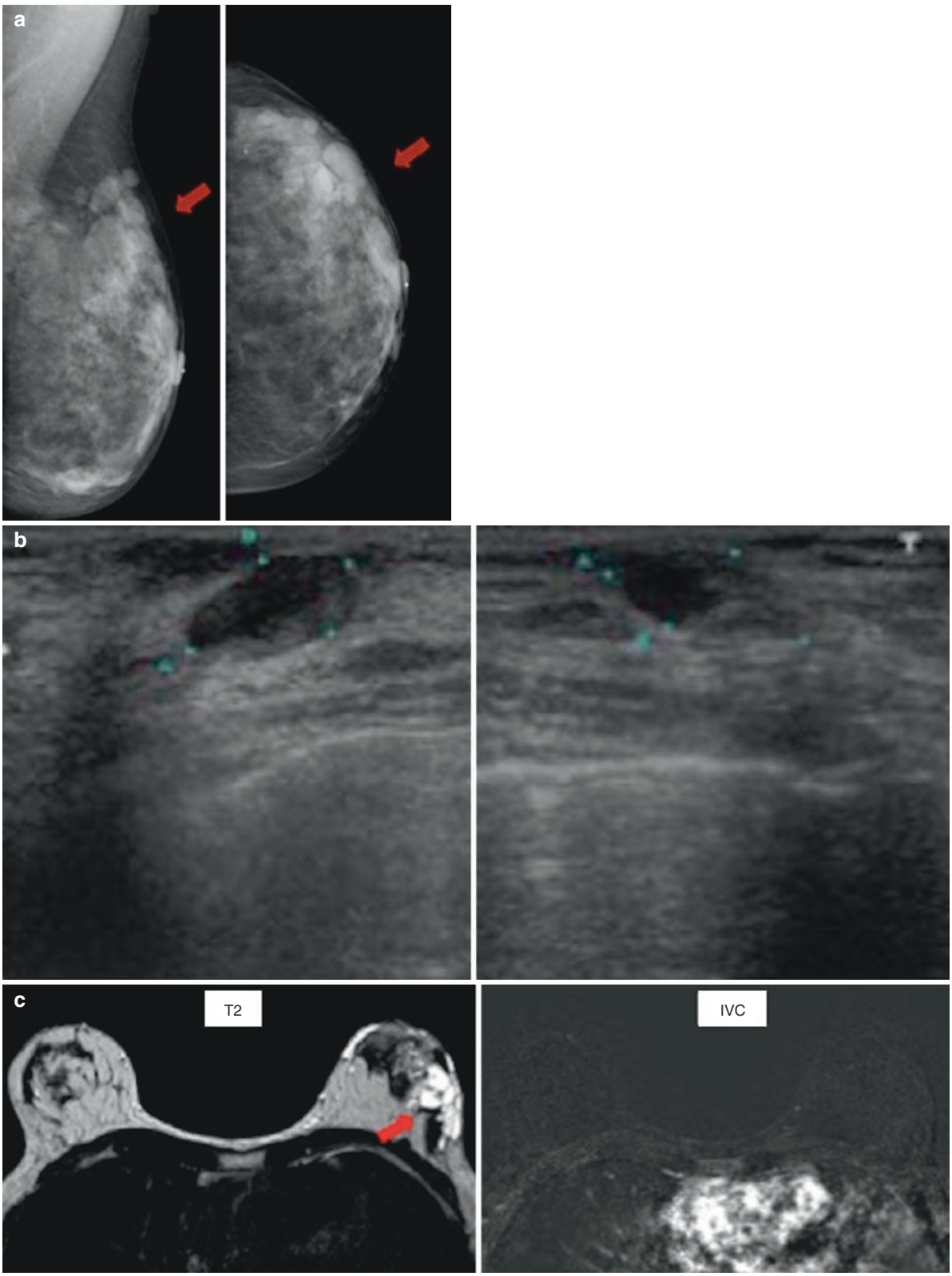

Fig. 21.38 Patient with free hyaluronic acid injection who had multiple palpable lumps at follow-up. (**a**) Mammogram of left breast (oblique and craniocaudal views): multiple nodular radiodense images, mostly located in the UOQ (*red arrow*), with some ill-defined margins and diffuse increased parenchymal density in the area. (**b**) Sonographic images: the mammographic nodules correspond to multiple nodular lesions (with complicated cystic and solid appearance) with uncircumscribed margins. (**c**) Breast MRI shows multiple nodules with an identical signal intensity of the liquid, different from the silicone and showing no enhancement in dynamic sequences. In this case, breast MRI allowed doubtful findings on mammogram and ultrasound to be solved. Figure 21.38b appeared in the European Aesthetic Plastic Surgery Journal (number 12). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

cases where the nipple is not preserved. The aim is to achieve greater symmetry and similarity in texture and color with the contralateral NAC. It is usually performed as a separate step after breast reconstruction when postoperative changes have stabilized (usually 6–8 weeks). Skin and subcutaneous tissue flaps are used (usually labia minora and the contralateral NAC when a mastopexy is also performed). Sometimes, only the nipple is reconstructed while the areolar area is tattooed.

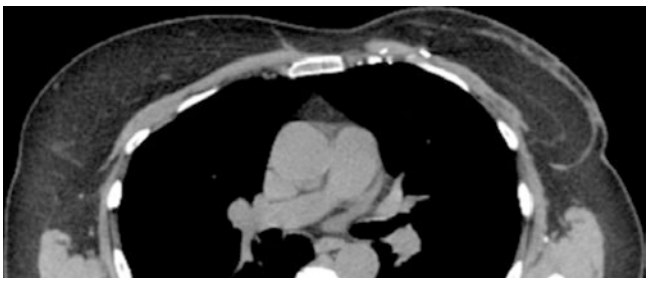

Fig. 21.39 Patient with left mastectomy and autologous reconstruction with DIEP flap. There is a contact line between the subcutaneous fat of the original breast and the flap fat. On CT there is a hyperdense line with variable thickness

21.4.1 Autologous Reconstruction

21.4.1.1 Radiological Tests

Physical examination and ultrasound are useful in the follow-up of patients who have undergone mastectomy without reconstruction. Nevertheless, they are less sensitive in patients who have undergone autologous reconstruction. Many tumor recurrences are located deep in the flap so they are not palpable. Reconstruction also decreases the contrast between the lesion (hypoechoic) and fat surrounding (hypoechoic too), rendering ultrasound ineffective. Frequent scars and fat necrosis often make it difficult to assess possible lesions with ultrasound and can produce hard palpable lumps that complicate the physical examination.

Mammography is useful in evaluating calcifications or microcalcifications by fat necrosis. It is also useful in detecting recurrence as it will increase the contrast between the flap (hypodense or fat) and possible recurrence (hyperdense).

Although breast MRI requires IVC administration, it allows the physician to distinguish benign tissue from recurrence in many cases because it can demonstrate fat inside the lesion (fat necrosis) or absence of enhancement after administration of IVC.

21.4.1.2 Radiological Findings

1. Distinguishing different types of flaps on imaging:

It is important to be able to differentiate different types of flaps on imaging, since clinical information often does not specify what type the patient has. Fat is key to recognizing an autologous flap in general. All flaps have in common the transfer of skin and fat and therefore, a fatty breast will be seen in all radiological tests. Muscle indicates a myocutaneous flap. The muscle is seen as a soft tissue density although there are atrophic changes over time and its appearance becomes more fatty. Vascularization is key to recognizing a muscle-sparing free flap with vascular anastomosis. Vascularization is assessed on breast MRI. The internal mammary region must be observed since this is the place where anastomosis was done and is sometimes the only sign of the presence of a flap or previous failed flap. It is important to note if there is IVC in the transferred vessels as this is a sign of viability.

There is a contact line formed between subcutaneous fat of the native breast and the fat transferred (Fig. 21.39). It is possible to distinguish three types of lines depending on its thickness: type 1, thickness smaller than 1 mm (almost not visible); type 2, thickness from 1 to 3 mm; and type 3, thicker than 3 mm. If the line increases in thickness after a month of surgery, then a recurrence, infection, or inflammation should be considered.

Characteristics of the main flaps:

- *Pedicled TRAM flap* (Fig. 21.40): Fatty breast is seen. Muscle density is seen anteriorly to the chest wall in a triangular shape. On breast MRI, the muscle density can be followed distally going to the abdomen (usually contralateral hemi-abdomen). In abdominal slices (CT or MRI), the absence of one rectus abdominis muscle and signs of postsurgical changes (metallic clips) can be seen.
- *Free TRAM flap* (Fig. 21.41): Fatty breast is seen. As in the pedicled TRAM flap, a muscle density is seen anteriorly to the chest although when there is atrophy this is not possible. The muscular density does not continue to the abdomen. In abdominal slices (CT or MRI), only a partial defect of the rectus muscle is seen. Postsurgical changes in the mammary internal region are seen because of vascular anastomosis in that area.
- *DIEP flap* (Fig. 21.42): Fatty breast is seen. There is no muscle density because there is no muscle transferred. In abdominal slices, the rectus muscle is complete although postsurgical changes can be seen as there are metallic clips inside it due to the harvesting of perforating branches of deep epigastric artery. Postsurgical changes are also seen in the internal mammary chain because of vascular anastomosis.
- *SIEA flap*: Fatty breast is seen. There is no muscle density because there is no muscle transferred. In abdominal slices, a complete and intact rectus muscle is seen, without any postsurgical change.

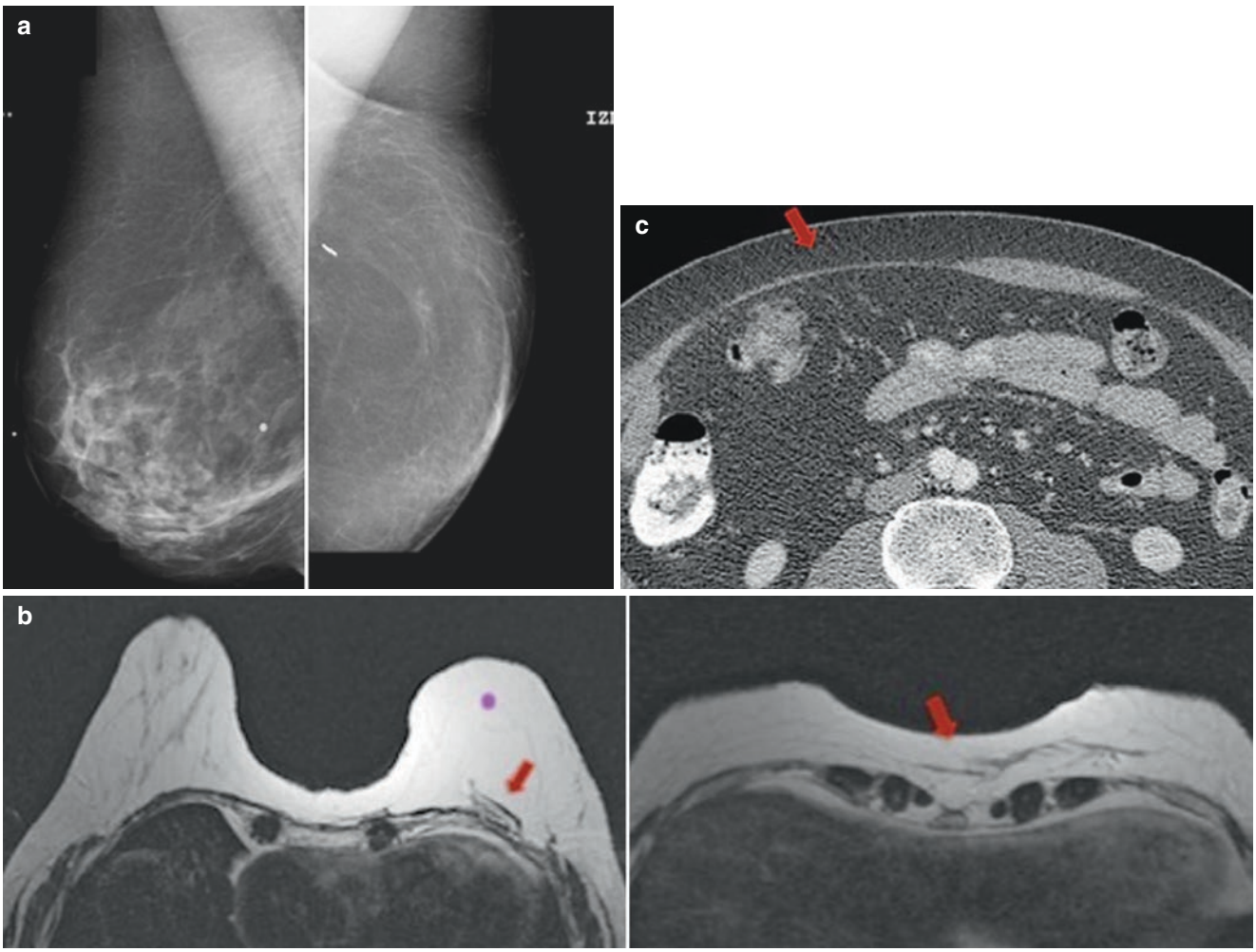

Fig. 21.40 Patient with left mastectomy and autologous reconstruction with pedicled TRAM. (**a**) On mammogram, a left fatty breast and the presence of metallic clips are indicating the presence of an autologous reconstruction. (**b**) Breast MRI with axial T2 sequence in two different slices: In the more superior slice, a left fatty breast (*purple dot*) is confirmed, and there is a muscle structure (*red arrow*) just beside to the anterior chest and the pectoralis muscle wall corresponding to a muscle with some hyperechoic lines inside relating to fatty atrophy. In the lower slice, the muscular density crosses the midline going to the abdomen. That is the key to knowing it is a pedicled TRAM flap. (**c**) Abdominal CT with IVC administration shows the absence of the right rectus abdominis muscle confirming the pedicled TRAM

- *LDM flap* (Fig. 21.43): Fatty breast is seen. The muscle density is seen as a band that comes from the back and parallel to the chest wall. When it is combined with an implant, it is usually seen posterior to the muscle band and is easily recognized on mammogram and the other tests. However, when there is only a partial defect or when it is not combined with an implant, it may be more difficult to recognize it on mammogram if the type of reconstruction is unknown. MRI will be useful (Fig. 21.13).
- *TDAP flap* (Fig. 21.44): Fatty breast is seen. There is no muscle density because there is no muscle transferred. Some vessels can be identified going to the back, related to the thoracodorsal pedicle.

It is important to examine all slices (from the most proximal to the most distal or superior abdominal slices). Special emphasis should be placed on these areas: the internal mammary chain (if there are postoperative changes, then there was microsurgery followed by a free TRAM, DIEP, SIEA, TUG, SGAP, or IGAP flap), the neobreast (if there is a muscle density, then there is a TRAM or LDM flap), the lateral chest wall looking at the latissimus dorsi muscle or its vascular pedicle (which indicates a LDM or TDAP flap), and finally the lower slices (if there is a muscle band, then there is a TRAM flap). All these are important especially in cases where a second flap has to be done because of a previous failure of another flap. It is possible to see a transferred LDM flap and postsurgical changes in the internal mammary chain, which means a previously failed DIEP or free TRAM flap. Another possibility is seeing a transferred LDM flap without an implant and asymmetry in relation to the contralateral breast suggesting complication and removal of the implant

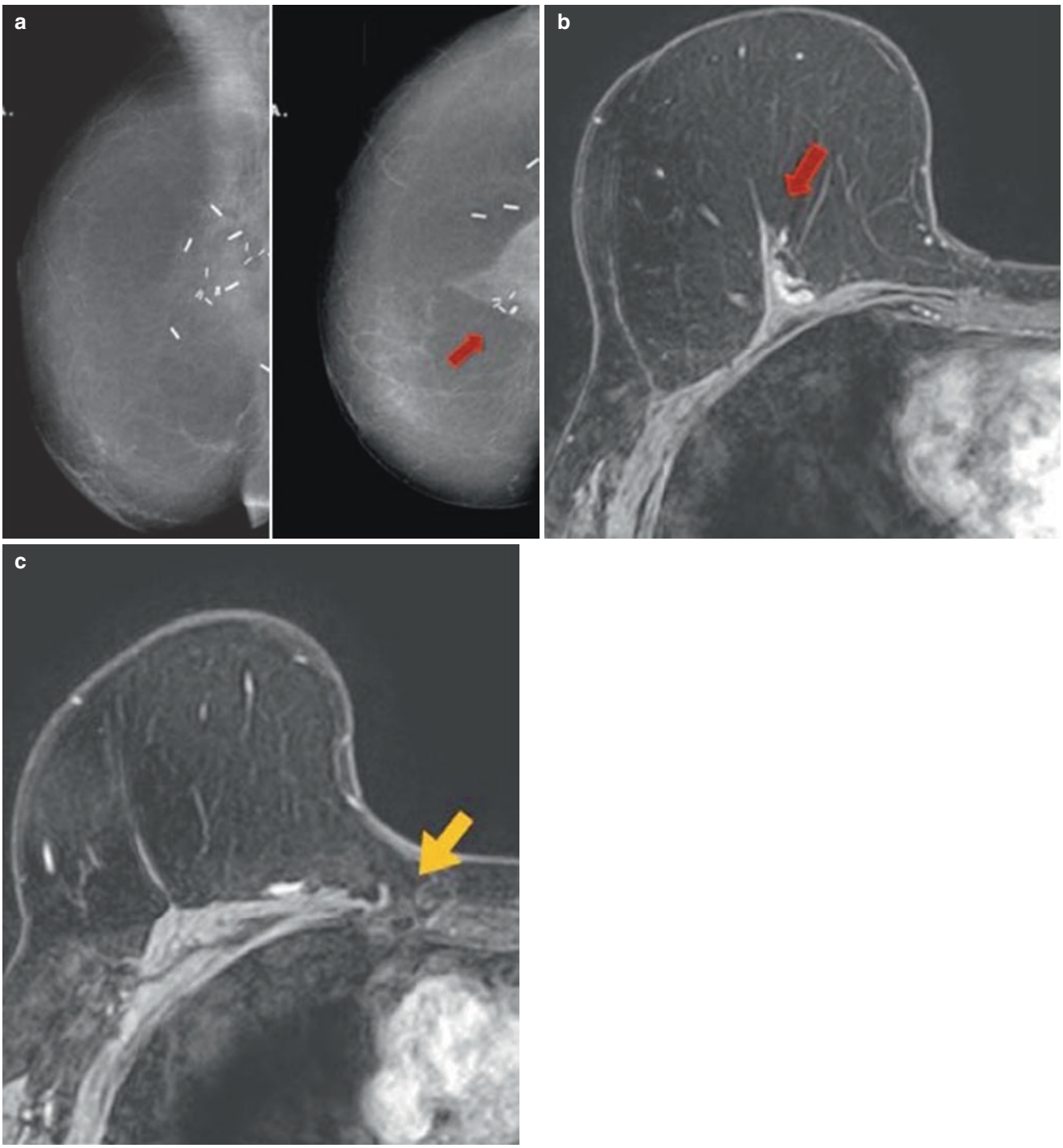

Fig. 21.41 Patient with right mastectomy and autologous reconstruction with free TRAM flap. (**a**) On mammogram, a muscular density is seen anteriorly to the chest wall and the pectoral muscle (*red arrow*). (**b**) Breast MRI with dynamic sequences showing the muscle transferred anteriorly to the pectoral muscle, with metallic clips and some vessels indicating good viability of the flap. (**c**) Breast MRI with dynamic sequences in an upper slice, at the level of the internal mammary vessels. The postoperative changes in the internal mammary vessels (*yellow arrow*) indicate the presence of vascular anastomosis and the presence of a free TRAM flap

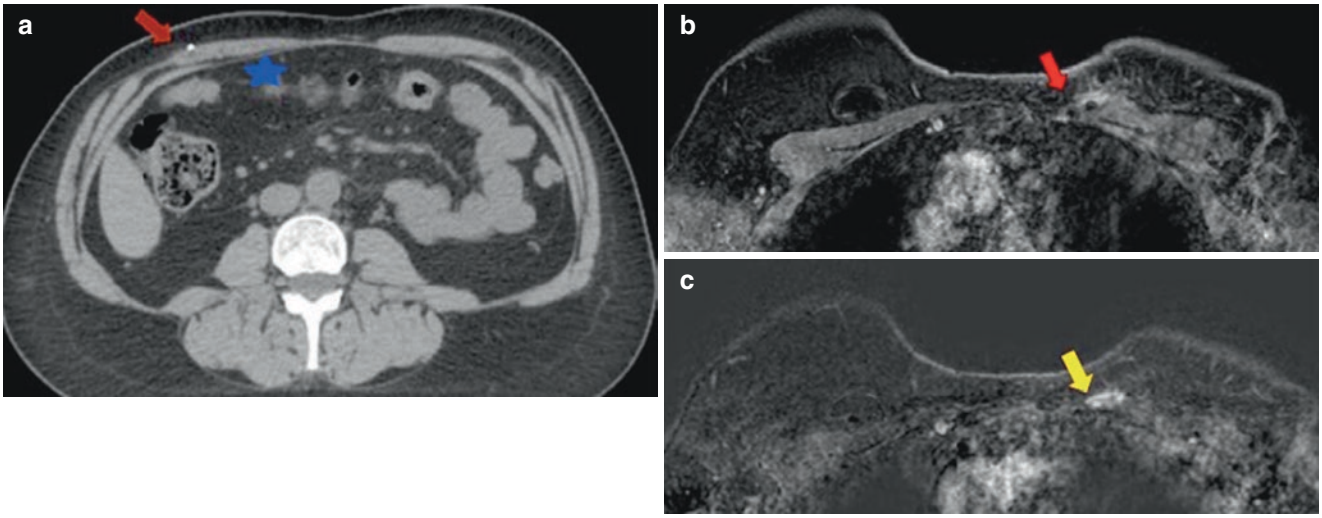

Fig. 21.42 Patient with left mastectomy and autologous reconstruction with DIEP flap. (**a**) Abdominal CT shows metallic clips (*red arrow*) on the rectus abdominis muscle indicating postoperative changes. However, the muscle is complete so it is not a free TRAM flap but a DIEP flap (*blue star*). (**b**) Dynamic sequence breast MRI shows metallic clips (*red arrow*) in the area of the internal mammary chain indicating the presence of vascular anastomosis. (**c**) Axial first subtraction sequence shows good vessel enhancement (*yellow arrow*) in the anastomosis, indicating good viability of the flap

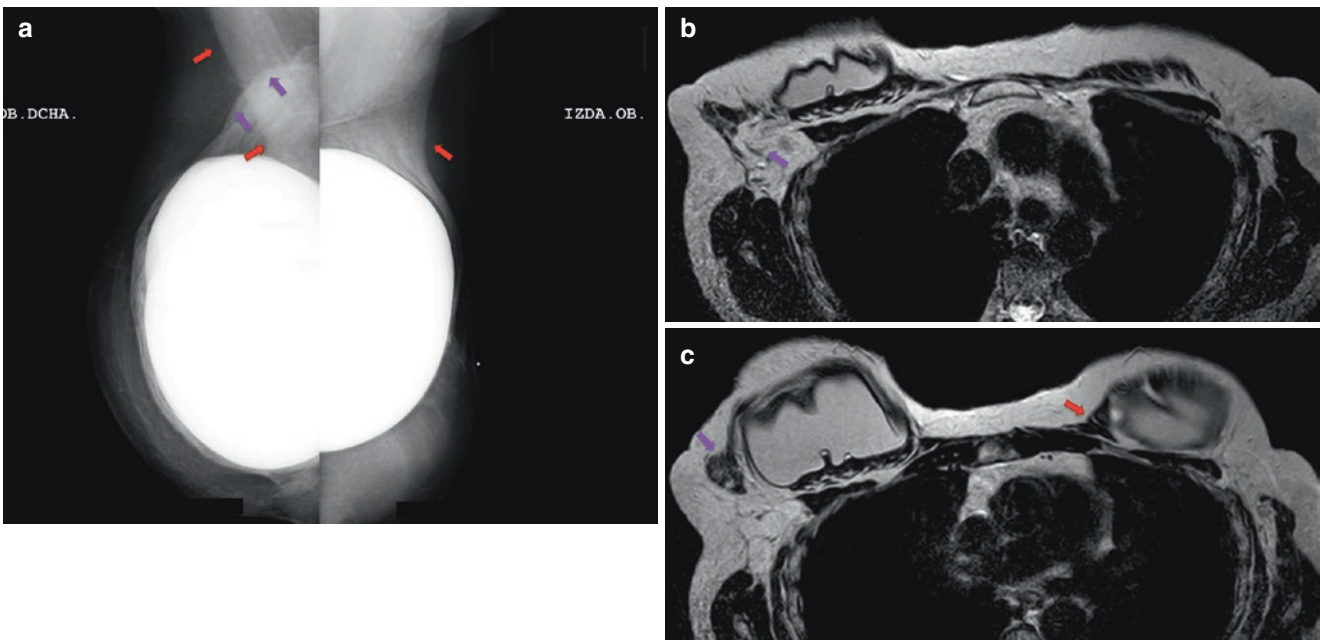

Fig. 21.43 Patient with bilateral mastectomy who had autologous reconstruction with an implant and LDM flap in the right breast and heterologous reconstruction in the left breast. The patient presented with a right axillary palpable lesion. (**a**) Mammography with oblique projections of both breasts: Both implants can be seen. In the left breast, the implant is located posteriorly to the pectoralis muscle (*red arrow*). In the right breast, the implant is located anteriorly; soft tissue visualized corresponds to the LD muscle (*purple arrow*) and the palpable area corresponds to the prominent and thick LD muscle containing fat just below. (**b**) and (**c**) Breast MRI T2 sequences confirm the presence of the pectoral muscle (*red arrow*) (posteriorly to the implant in the right breast and anteriorly in the left breast) and the LD muscle coming from the back and located anteriorly to the implant in the right breast making a prominence in the axillary region

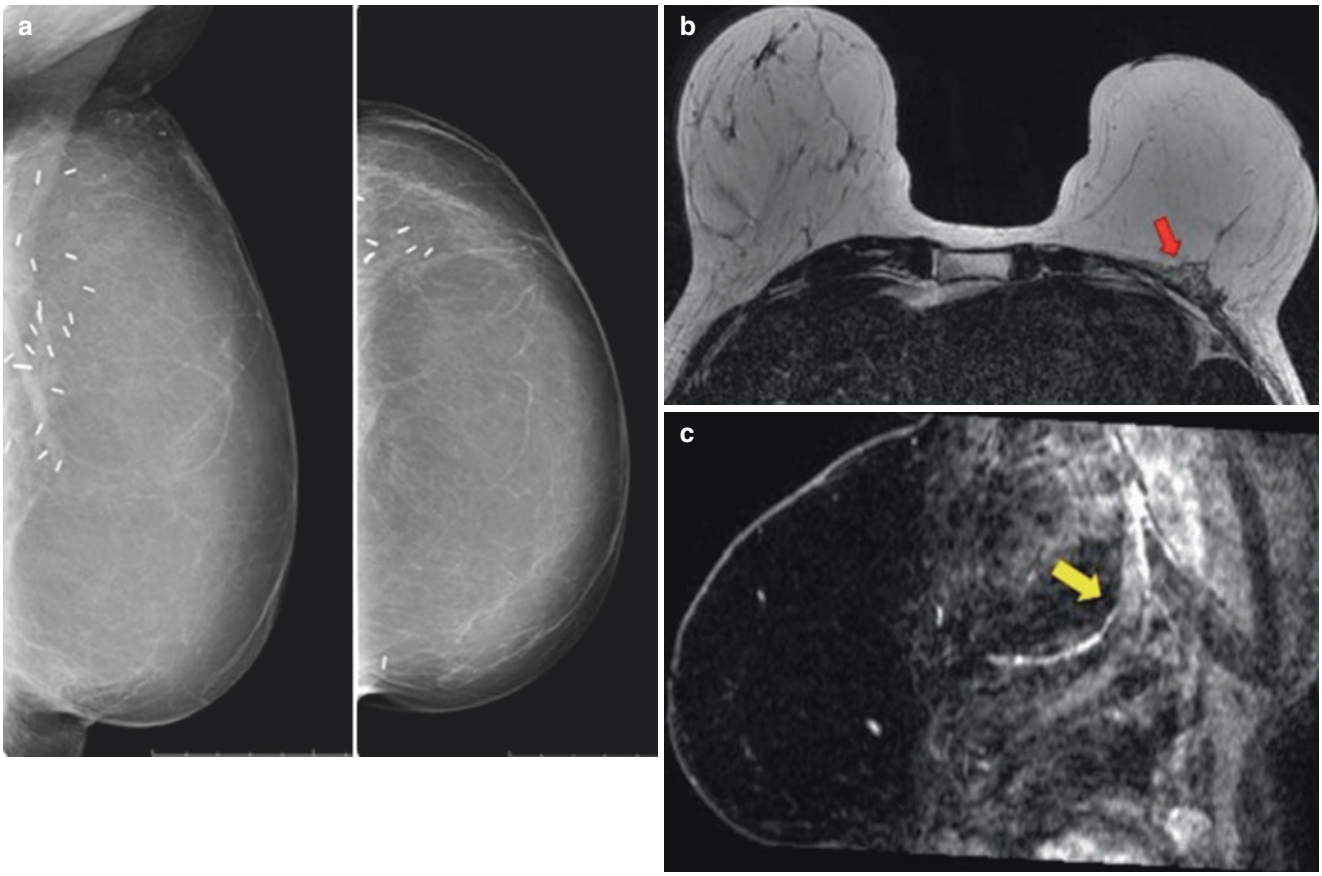

Fig. 21.44 Patient with left mastectomy. The patient had a failed autologous reconstruction with pedicle TRAM flap and later underwent a new autologous reconstruction with TDAP flap. (**a**) Mammogram shows a fatty breast with metallic clips that suggested a reconstructed breast. (**b**) Breast MRI with T2 sequence confirms the presence of a fatty and reconstructed breast with a muscular structure anteriorly to the pectoral muscle suggesting a TRAM flap (*red arrow*). (**c**) However, on a sagittal reconstruction of a dynamic sequence, a vascular structure (*yellow arrow*) is seen coming from the back corresponding to a perforating branch of the thoracodorsal artery and without any accompanying muscle, indicating a TDAP flap

(Fig. 21.45). An LDM or TDAP flap with perforating vessels going to the back and a muscle density in the deep of the neobreast indicates a previously failed TRAM flap and a second attempt with an LDM flap or a TDAP flap (Fig. 21.44).

2. Assessing complications of the neobreast:

Fat necrosis is one of the most common complications, especially with a pedicled TRAM flap. It is often caused by problems in the vascularization of the flap. Clinically it is suspected because the patient notes a new, hard, and palpable lesion, close to the scar. It is easily confused with recurrence. Radiologically, it is said to be a great imitator of cancer and complicates differential diagnosis. On mammogram, calcifications (from benign to punctate or pleomorphic calcifications) (Fig. 21.46), dense masses with irregular margins or even distortion images can be seen. On ultrasound, a wide spectrum of findings can be seen, from typically benign oil cysts (which are often mistaken for simple cysts) or hyperechoic lesions because of the presence of fat to hypoechoic, solid, and poorly defined lesions (Fig. 21.47). On breast MRI, a wide spectrum of findings can also be seen: typical image of an oil cyst (well-defined, oval, or round lesion hypointense on T2), a fat-containing solid lesion (hyperintense on both T1 and T1 sequences, suppressed on fat suppressed images that may or may not present peripheral or eccentric enhancement after IVC) (Figs. 21.46 and 21.49), or an irregular lesion with suspicious enhancement after IVC (Fig. 21.47). Conventional tests are enough to diagnose typically benign findings, whereas a breast MRI and/or biopsy are needed to diagnose BI-RADS® 4 and 5 lesions.

Venous congestion (Fig. 21.48) occurs especially in cases where vascular anastomosis is required. Mammographically, a diffuse increase density is seen (whereas usually a low or hypodense density is presented because of fat). On ultrasound, cutaneous and trabecular thickening and diffuse increased echogenicity of fat are seen. On breast MRI, edema and a diffuse increased enhancement related to inflammatory changes are seen.

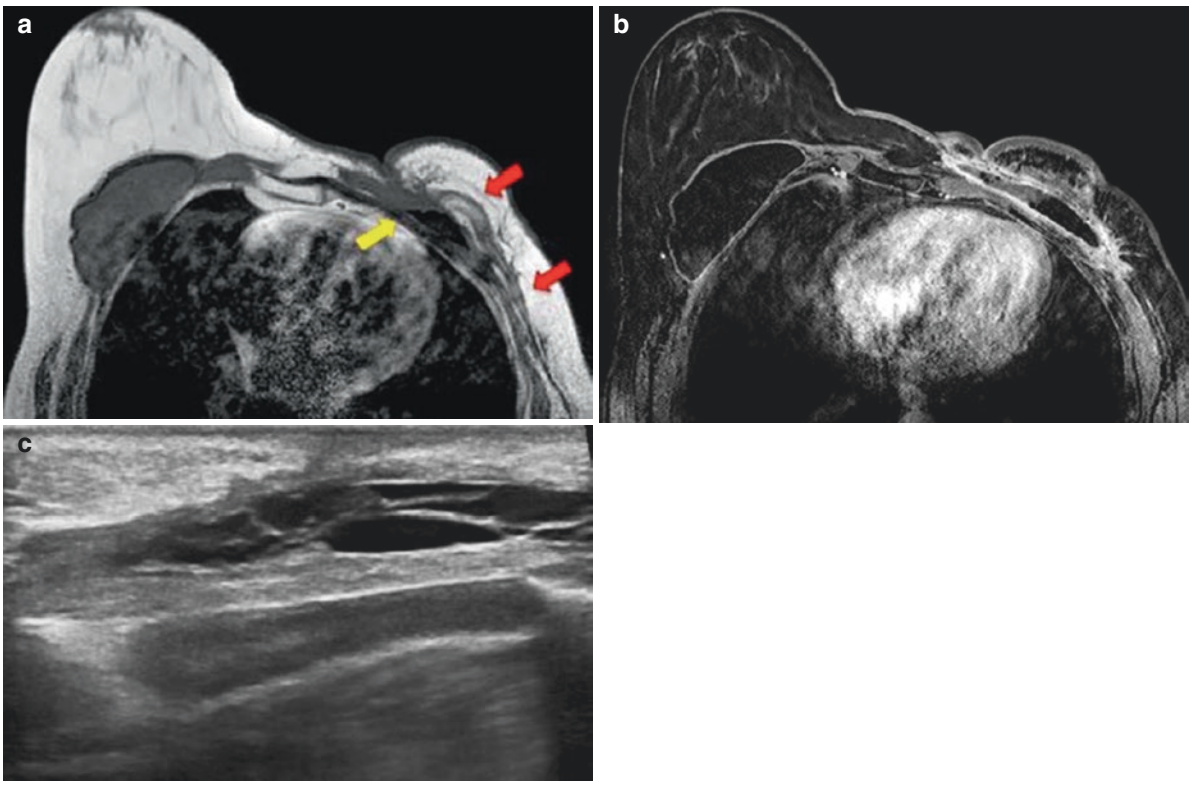

Fig. 21.45 Patient with left mastectomy and failed autologous reconstruction with LDM flap and implant. Due to recurrent peri-prosthetic infection, the implant was eventually removed but the transferred LD stayed. (**a**) Breast MRI with T1 sequences shows the LDM flap (*red arrow*) coming from the back and located anteriorly to the pectoral muscle and also anteriorly to a complex and fluid collection (*yellow arrow*) relating to residual abscess. (**b**) The dynamic sequence shows a thick peripheral enhancement around the collection suggesting infection. (c) Ultrasound shows a tabicated fluid collection; an FNA was performed to obtain an antibiogram

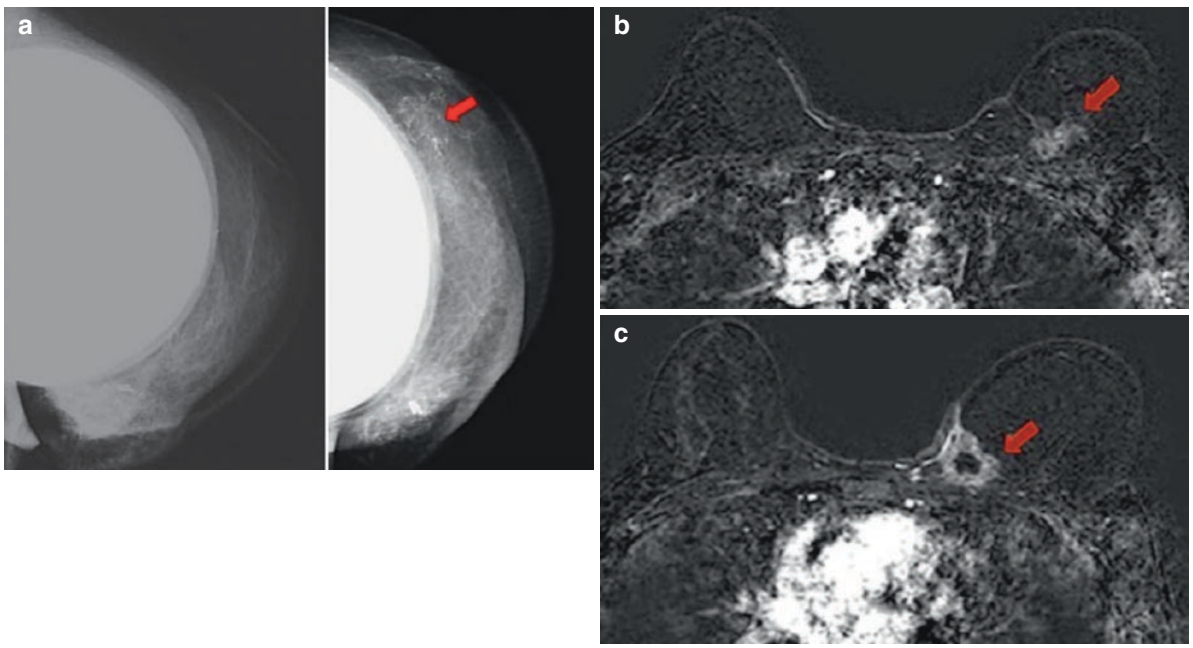

Fig. 21.46 Patient with left mastectomy and autologous reconstruction with LDM flap and implant. The patient had capsular contracture and also noted a new palpable lump in the outer quadrants of the left breast underlying scar. (**a**) Craniocaudal and Eklund projections of the left breast: When the implant was pushed back, a group of new pleomorphic calcifications (*red arrow*) is seen in the outer quadrants that correlated with the palpable area. Those calcifications would be suspicious but a breast MRI was performed. (**b**) A dynamic sequence in a superior slice shows the presence of heterogeneous and ill-defined enhancement in that area. (**c**) In a lower slice, the lesion shows a fat center, indicating fat necrosis. Biopsy was not recommended. Figure 21.46a appeared in the European Aesthetic Plastic Surgery Journal (number 14) and Figures 21.46b and 21.46c appeared in the European Aesthetic Plastic Surgery Journal (number 13). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

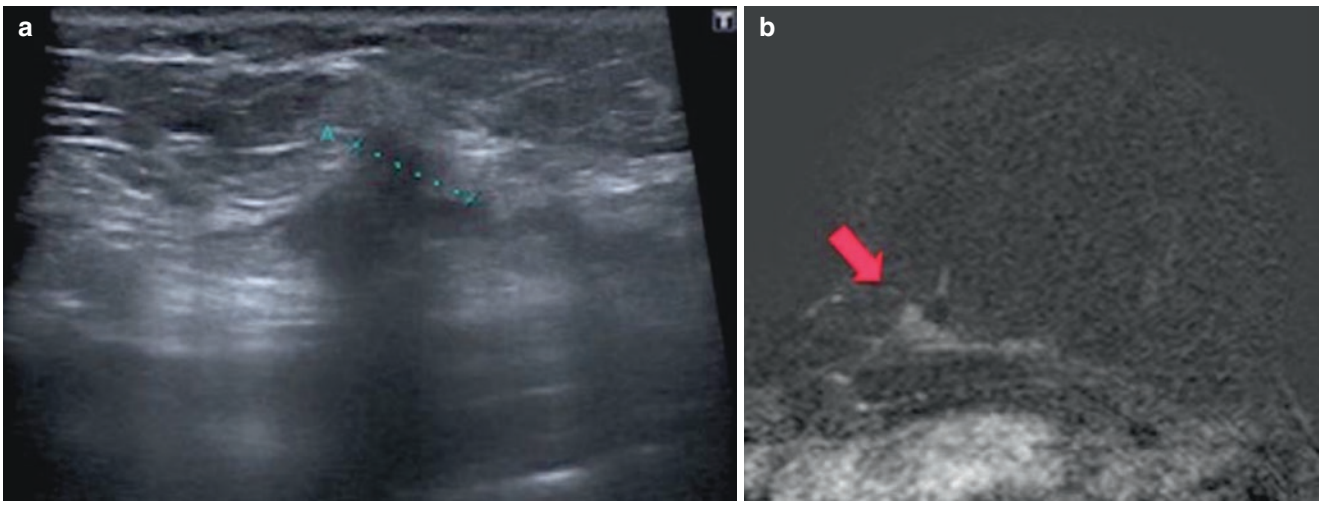

Fig. 21.47 Patient with left mastectomy and autologous reconstruction with DIEP flap. The patient had a superficial, palpable lesion in the UIQ of the left breast. (**a**) Ultrasonography shows a suspicious irregular hypoechoic and poorly defined lesion, with posterior acoustic shadowing. (**b**) Subtracted image after IVC administration: An irregular and ill-defined lesion in that area is seen without a fat center. A core biopsy was performed with fat necrosis result. In this case, the biopsy was necessary to demonstrate the fat necrosis

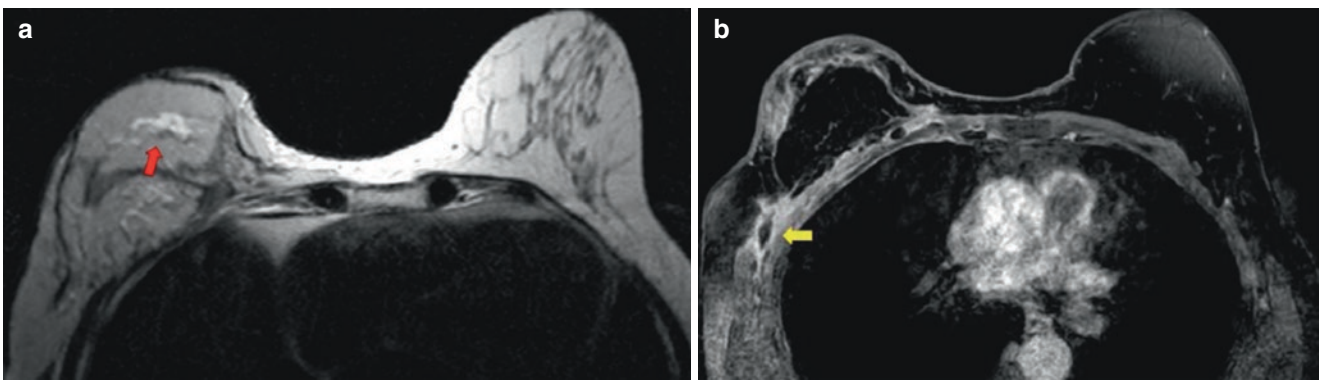

Fig. 21.48 Patient with right mastectomy and autologous reconstruction with DIEP flap who had complications with congestive changes. (**a**) Breast MRI T2 sequence shows edema, cutaneous and trabecular thickening, and fluid collection (*red arrow*). (**b**) Dynamic sequence after IVC administration shows irregular enhancement, predominantly in the periphery of the flap, in the periphery of an organized fluid collection beside the flap (*yellow arrow*), and in the vascular areas. The appearance in some slices looked like an implant. This image originally appeared in the European Aesthetic Plastic Surgery Journal (number 14). Reproduced with permission from the Asociación Española de Cirugía Estética Plástica

Fluid collections (Fig. 21.49) are usually related to postoperative seroma with simple cyst appearance. Sometimes a complicated or complex cyst can be seen, and then the possibility of bleeding, secondary infection, or abscess (Fig. 21.45) should be considered.

Imaging can also assess tissue necrosis and wound closure failure.

21.4.1.3 Follow-Up Protocol

The possibility of recurrence after breast-conserving surgery and mastectomy is low. In autologous reconstruction, the recurrences are located predominantly in two areas:

- *Superficial zone*: in the contact line between the flap and the subcutaneous fat of the native breast. It is usually detected by physical examination although sometimes it can be an incidental finding in a screening test (Fig. 21.50). On imaging, special attention should be paid to the variations and increases of thickness of that line that are not justified by infection or inflammation.
- *Deep zone*: in the posterior margin of the bed mastectomy, typically along the pectoralis major muscle, deep to the flap. Given its deep location, it is not usually detected by physical examination.

Since the incidence of recurrence is so low, there is debate about whether follow-up should be performed in these patients. However, possible screening with annual mammography and ultrasound has been suggested, relegating breast MRI to evaluating possible complications or indeterminate findings on conventional tests.

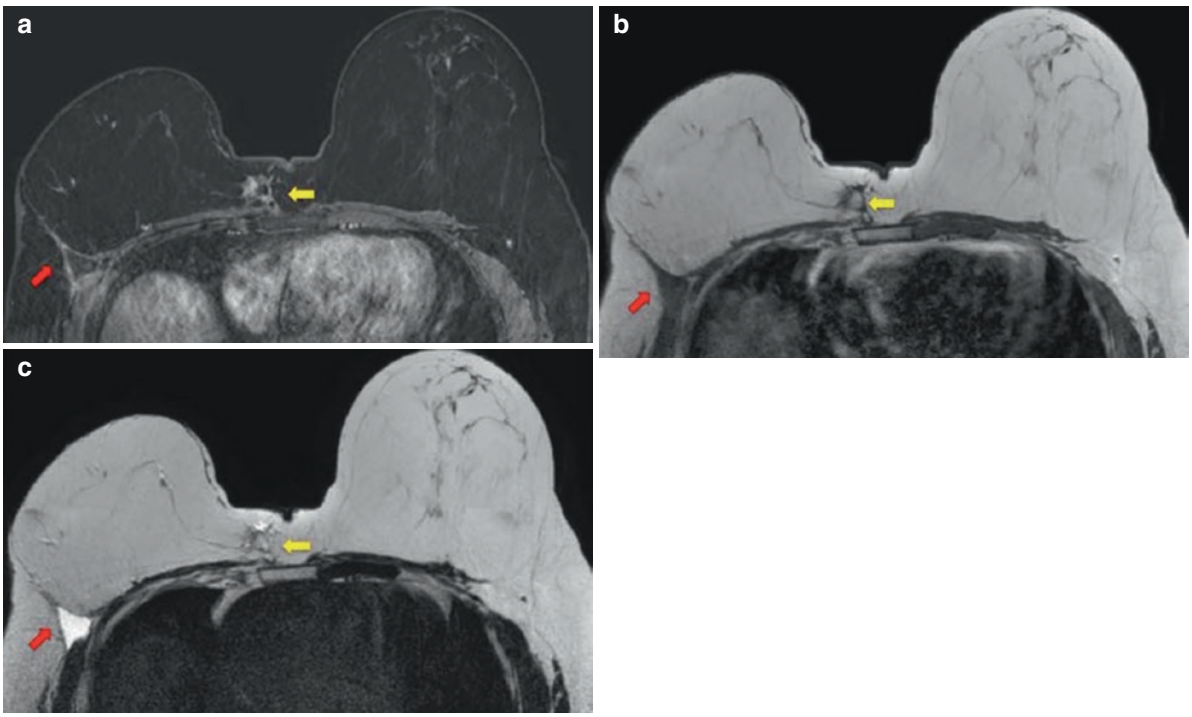

Fig. 21.49 Patient with right mastectomy who had a new palpable lesion in the inner quadrants. (**a**) Breast MRI dynamic shows a fluid collection in the outer quadrants (*red arrow*) with thin and low enhancement and an irregular lesion in the inner quadrants (*yellow arrow*) relating to the palpable lesion. (**b**) and (**c**) T1 and T2 sequences confirm the presence of a simple fluid collection relating to seroma in the outer quadrants and in the inner quadrants the palpable lesion has fat inside indicating fat necrosis. For that reason, biopsy was not necessary

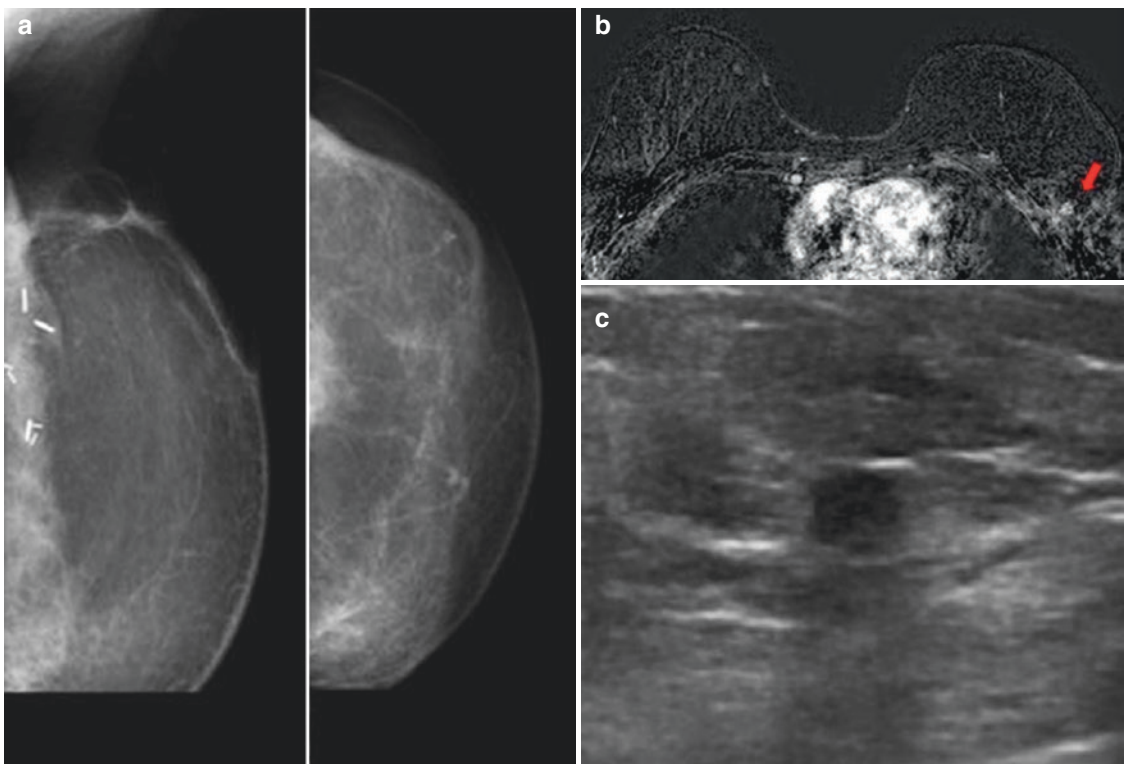

Fig. 21.50 Patient with left mastectomy and DIEP reconstruction with a millimetric recurrence visualized only on a follow-up MRI. (**a**) Left mammography shows a fatty breast without suspicious findings. (**b**) Breast MRI subtracted image shows a small, irregular and ill-defined lesion located in the contact area of the flap with the native breast in the outer quadrants, in the periphery of the flap (*red arrow*). (**c**) Second look ultrasound was performed; a millimetric lesion was found and biopsied with the result of invasive ductal carcinoma, confirming recurrence

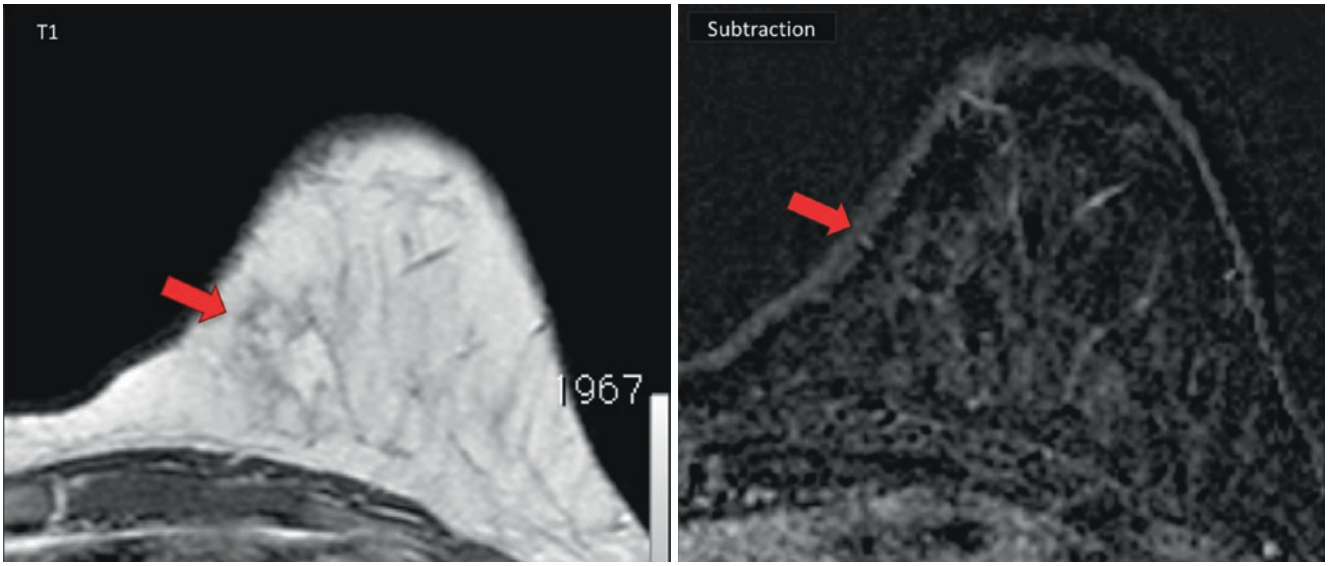

Fig. 21.51 Patient with a personal history of lipofilling for aesthetic reasons. After some time, she presented with a palpable lump at that location. However, conventional imaging tests (mammography and ultrasound) were completely normal. For that reason, an MRI was performed. Only a slight fat trabeculation in that area was seen, without any lesion and with no enhancement (*red arrow*), because the injected fat and the normal fat of the breast fused together. In cases like this where the patient's personal history is unknown, the radiological finding would be a BI-RADS® 1

21.4.2 Lipofilling, Lipomodeling, or Free Fat Grafting

21.4.2.1 Radiological Tests and Findings

1. BI-RADS® 1 Identical to normal breast fat (Fig. 21.51): It is the ideal situation. It is common for the radiologist not to identify a breast with lipofilling if he does not know the patient's clinical history. On mammogram, free fat will mix with the normal fat not giving any especial finding; only the breast will appear as a less dense breast due to free fat, consequently allowing better detection of possible cancer because of increased contrast with a radiodense mass or calcifications. On ultrasound and breast MRI, it would also be indistinguishable from the rest of the mammary fat.
2. BI-RADS® 2 Benign findings (Fig. 21.52): Oil cyst is the most common manifestation when the lipofilling shows radiographic abnormalities. Benign calcifications (usually coarse or rim calcifications) can also be seen. The breast MRI usually shows solid lesions with a fat center, without enhancement or with rim and thin enhancement related to fat necrosis. For that reason, it is important to review T1, T2, and fat suppressed sequences.
3. BI-RADS® 3 Probably benign findings (Fig. 21.53): Round or oval, well-defined solid lesions with type I enhancement curve or a new complicated cyst.
4. BI-RADS® 4 Suspicious findings (Fig. 21.54) especially due to fat necrosis. On mammogram, a distortion image or suspicious calcifications can be seen. On ultrasound, irregular solid lesions with posterior shadowing can be noticed and on MRI lesions with type II or III enhancement curves can be found.

21.4.2.2 Follow-Up Protocol

There is no established protocol because its use has increased only rather recently. Although it seems proven that this technique is not associated with an increase of breast cancer, there are still no conclusive and statistically significant studies regarding its association with complications and with radiological findings. For that reason, if it is possible, it is recommended to have a mammogram and/or ultrasound prior to the lipofilling. Some authors have even recommended performing a baseline mammogram 6 months after lipofilling to be able to assess posterior changes. However, many changes could happen after 6 months, for example, fat necrosis, and other findings change over time despite being benign. Additional tests and interventional procedures present a greater burden on the patient and increase anxiety and concern. For that reason, we try to distinguish two situations:

1. Symptomatic patient with a new palpable lesion: The mammogram and ultrasound should be performed. The breast MRI and/or biopsy may be performed if previous tests are inconclusive or if the patient has a personal history of breast cancer or high risk or family history.
2. Asymptomatic patient: annual mammogram ± ultrasound. Other option is to perform a mammogram and ultrasound 6 months after injection as baseline tests and later performing other tests if there are changes.

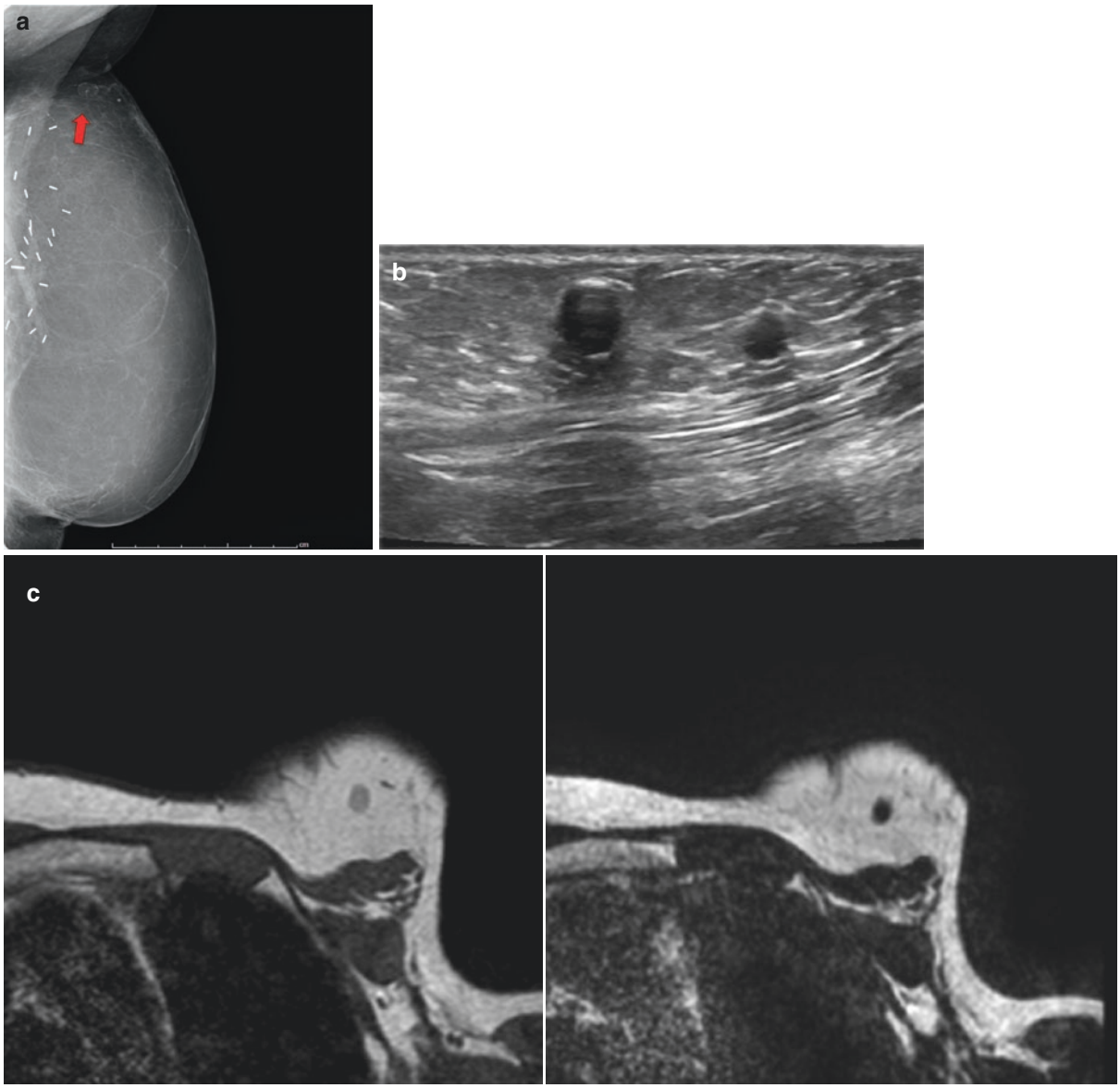

Fig. 21.52 Patient with left mastectomy and autologous reconstruction with a DIEP flap and lipofilling injection a second time in upper quadrants to fill a small defect. The patient presented with a new palpable lump at some time after injection. Mammogram, ultrasound, and MRI were performed, and a BI-RADS® 2 classification was made. (**a**) Oblique view of left mammogram only shows rim calcification related to the palpable lesion. (**b**) Ultrasound shows several lesions with simple cystic appearance. Although appearing as simple cysts they were really oil cysts as MR confirmed, being hypointense on T2, iso-/hyperintense on T1 and with no enhancement after IVC administration (**c**)

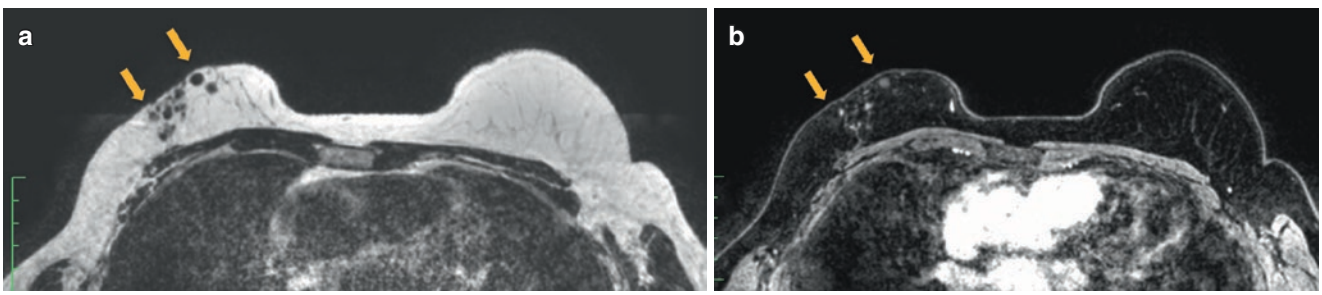

Fig. 21.53 Patient with right breast-conserving surgery and lipofilling in UOQ. The patient presented with some new palpable lumps in that area after lipofilling (*yellow arrows*). MRI shows (**a**) several round lesions, most of them well defined, hypoechoic on T2 and (**b**) with a soft enhancement with type I curve on subtracted image. With those characteristics, it was classified as BI-RADS® 3, and a follow-up was recommended

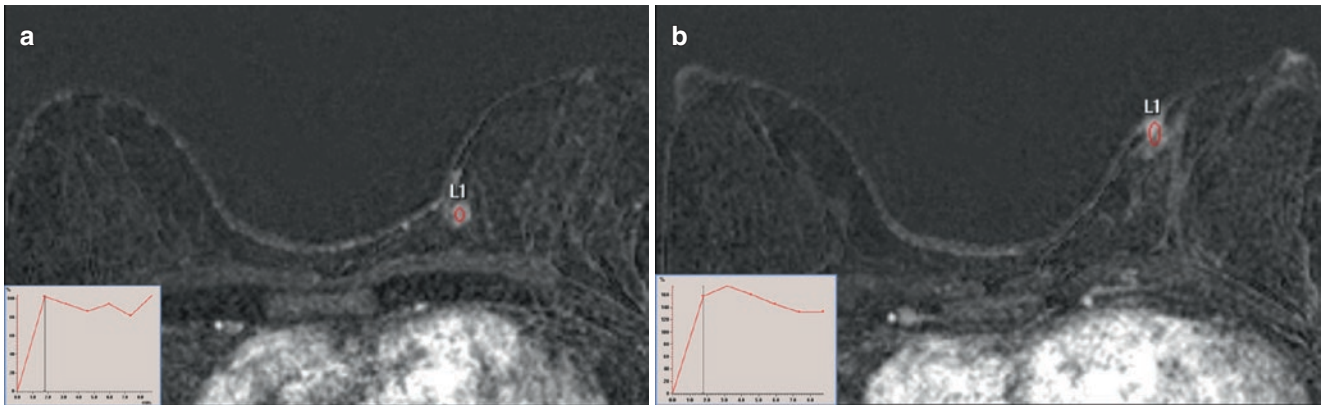

Fig. 21.54 Patient with a history of breast-conserving surgery in the UIQ of left breast with a lipofilling injection in that area. The mammogram did not show any significant finding, only metallic clips. Ultrasound showed several lesions with solid or complicated cystic appearance, but some of them had ill-defined margins. For that reason, MRI was performed. (**a**) One of the lesions shows well-defined margins but a type III curve. (**b**) Another lesion shows spiculated and irregular margins and a type III curve. Therefore, the lesions were classified as BI-RADS® 4, and cytology was performed with the result of fat necrosis

21.4.3 NAC Reconstruction

21.4.3.1 Radiological Tests and Findings

Findings on imaging are not common, but sometimes, when the reconstruction is very fresh, the following can be observed:

1. On mammogram: a radiodense periareolar line can be seen.
2. On MRI: areola skin thickening that can be asymmetrical to the contralateral NAC. Like in mastopexy, periareolar changes can be appreciated especially in gradient echo and fat suppression sequences. Sometimes the new NAC can enhance especially if the reconstruction is recent, which must not be confused with a malignancy. Other times a magnetic artifact produced by the tattoo ink can be observed.

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Integrated Breast Biopsy for Best Radiological Diagnosis of Breast Cancer

22

Enrico Cassano and Chiara Trentin

22.1 Introduction

Breast biopsy (BB) is a useful tool for breast cancer diagnosis. It allows characterization of any breast lesion that is undefined at imaging.

In the presence of a known carcinoma, BB can be used to assess its biological features. Over the past years, knowledge of the biological features of breast cancer (e.g., estrogen and progesterone receptor expression and the presence of HER2) has become essential to establish treatment strategies.

22.1.1 Indications for Breast Biopsy

The lesions that need to be characterized are those considered suspicious for malignancy. The level of suspicion for malignancy is classified using the American College of Radiology Breast Imaging Reporting and Data System (ACR BI-RADS) which is valid for mammography, ultrasound, and MRI examinations [1].

BI-RADS classification is described in detail in Table 22.1.

Biopsy is indicated for lesions that are classified as BI-RADS 4 (2–95% chance of malignancy) and BI-RADS 5 (greater than 95% chance of malignancy).

Short-interval follow-up is recommended for BI-RADS 3 lesions (risk of malignancy ≤2%) unless there are valid clinical indications for biopsy or when follow-up with imaging is difficult or unreasonable to perform.

Table 22.1 ACR BI-RADS assessment categories

BI-RADS® assessment categories			
Category 0	*Mammography*: Incomplete—need additional imaging evaluation and/or prior mammograms for comparison		
	Ultrasound and MRI: Incomplete—need additional imaging evaluation		
Category 1	Negative		
Category 2	Benign		
Category 3	Probably benign		
Category 4	Suspicious	Mammography and ultrasound	Category 4A: Low suspicion for malignancy
			Category 4B: Moderate suspicion for malignancy
			Category 4C: High suspicion for malignancy
Category 5	Highly suggestive of malignancy		
Category 6	Known biopsy-proven malignancy		

22.2 Type of Biopsies

Biopsies can be guided by imaging (ultrasound, mammography, or MRI). Imaging guidance is essential for non-palpable breast lesions or for lesions that are deep within the breast tissue or mobile to guide the needle in the biopsy target.

There are three types of imaging guided biopsies:

- Fine needle aspiration (FNA)
- Automated large-core biopsy (CB)
- Vacuum-assisted biopsy (VAB)

The latter two are most commonly used on the breast because of their ability to sample greater amounts of tissue. FNA is still widely used in Europe and Asia.

There are several factors that help a doctor decide which type of biopsy to perform although there is still a certain degree of variability regarding the selection of biopsy and needle type.

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22.2.1 Fine Needle Aspiration (FNA)

Fine needle aspiration (FNA) is a popular and valuable tool in preoperative assessment of breast masses. It is the least invasive method of biopsy; it is relatively inexpensive and can be performed with little complications but allows for only cytological analysis.

This will allow to differentiate benign from malignant lesions without further characterization.

FNA is usually performed using fine (27–20-gauge) needles, to remove a sample of cells under ultrasound, or rarely X-ray guidance, without the need of local anesthetic injection.

Ultrasound-guided biopsy is performed with the patient in supine position or slightly tilted to the side.

The sampling can be done using the needle alone (for capillarity) or the needle put together with a syringe (manual suction) or with a distant suction system (manual suction or suction pump).

Usually, the radiologist inserts the needle into the lesion more than once (usually 2–4 times) in a plain coaxial to the transducer in order to visualize the needle's trajectory during the procedure.

Once the lesion is targeted, the radiologist twists and thrusts the needle back and forth within the lesion while keeping negative pressure on the syringe to collect material which is then sent to pathology to be analyzed.

FNA is generally used when the breast nodule is likely to be a fluid-filled cyst, for symptomatic cystic lesions and for suspicious lymph node analysis.

FNA can serve as a valid alternative to excisional biopsy when it is the only available tool to diagnose a malignant lesion due to limited hospital resources.

FNA is also the preferred biopsy type when other biopsy methods are not feasible due to technical reasons such as small breast size and lesions that are too close to the skin or too deep within the breast tissue or due to patient's contraindications such as patients with coagulopathies and patients that cannot discontinue anticoagulant therapy. In the absence of clinical/radiological suspicious findings, any non-bloody fluid aspirate from a cystic lesion (e.g., milk white, gray, yellow, blue, green) can be discarded. If sampled material is bloody, further cytological analysis is mandatory.

FNA has some limitations related to operator and cytopathologist experience.

There are instances where the differentiation of benign and malignant is not possible with FNA. This is most commonly due to paucity of specimen sampling or in cases where there is a morphological overlap between benign and malignant lesions (e.g., atypical hyperplasia and low-grade carcinoma in situ or papillary lesions).

It is difficult to differentiate in situ lesions from infiltrating cancers with FNA; inadequate results are more common that with other biopsy methods (up to 54%) and evaluation of microcalcifications is problematic [2].

Cytological diagnosis is more accurate in lesions with a high cellular component (such as invasive ductal carcinoma or metastatic lymph nodes). In the presence of areas with little cellular component (such as hyalinized fibroadenomas, fibrotic lesions, or infiltrating lobular cancers) or in cases with evidence of morphological overlap between benign and malignant lesions (e.g., atypical hyperplasia and low-grade carcinoma in situ or papillary lesions), some interpretation problems can occur. In order to overcome such problems, biopsy samples are classified according to the National Health Service Breast Screening Program (NHSBSP) into five categories: inadequate (C1), benign (C2), atypia probably benign (C3), suspicious of malignancy (C4), or malignant (C5) [3]. C3 and C4 categories of breast lesions require a second-line biopsy evaluation.

More recently it has become possible to further characterize cancers diagnosed by FNA using cell block immunohistochemistry (IHC) and other molecular studies. Fine needle aspiration samples and cell blocks can be collected simultaneously using a polyvinyl alcohol foam core device that attaches to a fine needle. When FNA sampling is finished, the specimen is ejected from the needle onto a slide by air pressure from an attached syringe; the device is then removed, placed in a formalin specimen pot, and sent for histopathology examination (Fig. 22.1) [4].

22.2.2 Automated Large-Core Biopsy (CB)

Automated large-core biopsy (CB) uses a spring-loaded semiautomatic guillotine needle device (Fig. 22.2). CB is performed under ultrasound guidance (rarely stereotactic). The needles used are 18–14-gauge needles. Each needle insertion gives a tissue sample; two to four needle passes are performed after injection of local anesthesia and skin incision.

During the procedure the patient lies in the supine position. The needle is introduced into the target along the direction of the long axis of the US probe selecting the most efficient, safe, and short trajectory (e.g., parallel to the chest wall to avoid possible pneumothorax). The needle is advanced until the tip is a few millimeters proximal to the edge of the lesion. The core biopsy gun is then fired for sampling. The needle is withdrawn and the specimen is transferred into a fixative (Fig. 22.3).

CB has been well accepted as a valid alternative to surgical biopsy, for its lower cost, increased patient comfort,

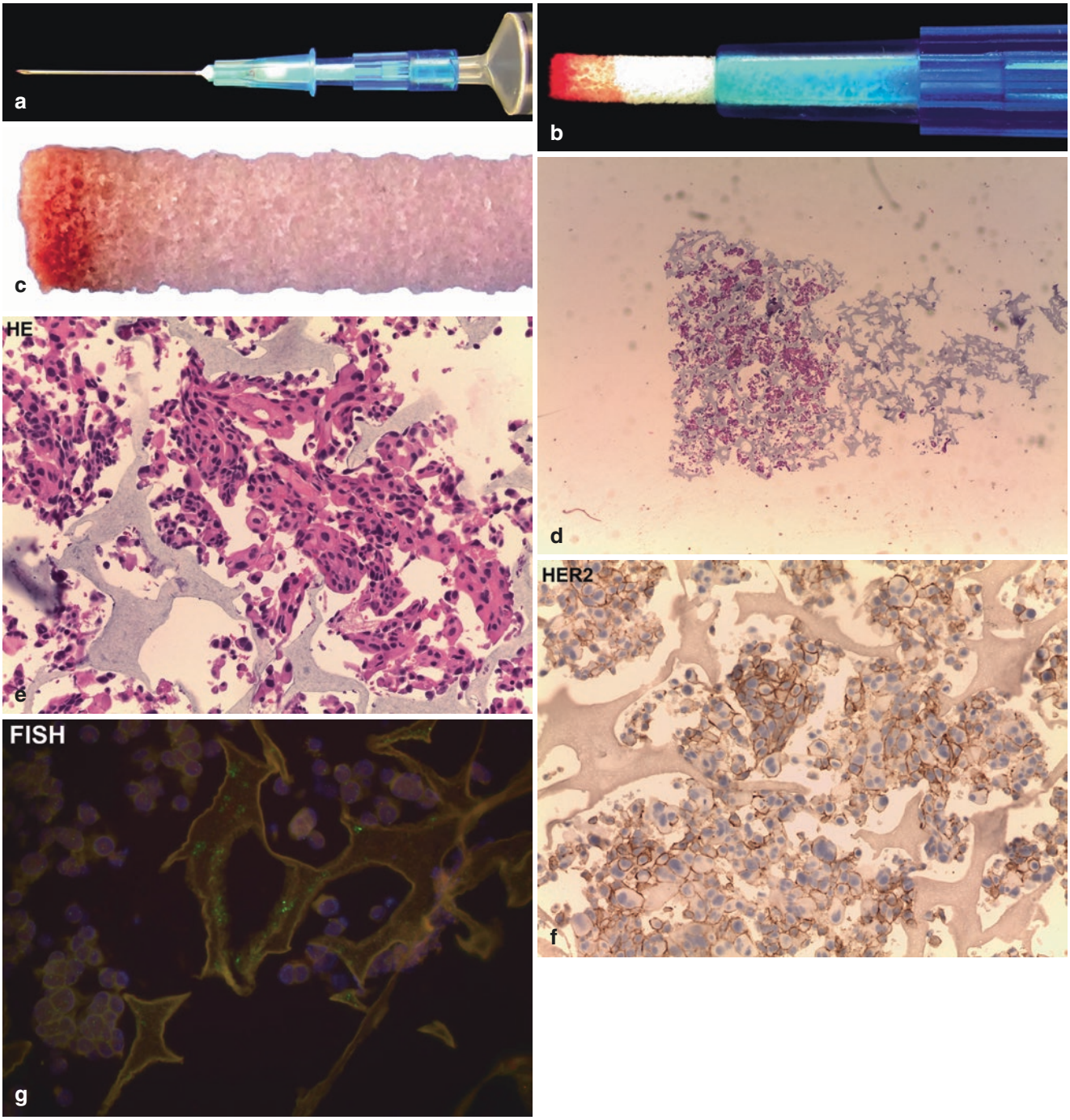

Fig. 22.1 (**a**)–(**c**) *CytoFoam device*: consists of a plastic adaptor that has a core of sterile polyvinyl alcohol (PVA) foam protruding from its lumen at the end that fits onto the hub of the needle. (**d**)–(**g**) After formalin fixation the PVA core is removed from the adapter, paraffin processed, and sectioned in the usual way, allowing histological assessment: hematoxylin-eosin, HER2, and FISH test (for courtesy of Dr G. Renne, IEO, Milan)

and short duration of the procedure. However, literature data reports some limitations related to the need of multiple needle insertions to obtain multiple specimens, technical difficulties related to small-sized lesions, difficulty of sampling microcalcifications, and risk of incomplete characterization for lesions with complex histology (such as lesions containing calcificatios, or atypical ductal hyperplasia and ductal carcinoma in situ (DCIS), or DCIS and invasive cancer) [5]. Also CB has a significant reported false-negative rate of 3–11% and underestimation rates of 16–56% [6, 7].

According to European guidelines [8] pathological analysis of CB specimens is divided into five categories: normal tissue (B1), benign lesion (B2), lesion of uncertain malignant potential (B3), lesion suspicious of malignancy (B4), and malignant lesion (B5).

B3 lesions include atypical epithelial proliferations (atypical ductal hyperplasia (ADH)), lobular neoplasias (LNs: atypical lobular hyperplasia (ALH), and lobular carcinoma in situ (LCIS)), papillary lesions, radial scar/complex sclerosing lesions, and phyllodes tumors.

22.2.3 Vacuum-Assisted Biopsy (VAB)

Vacuum-assisted biopsy (VAB) can be performed under X-ray, ultrasound, or MR guidance, depending on the target characteristics. Biopsy is performed under local anesthesia using larger needles (from 14 to 7 gauge), sampling a larger volume of tissue. The needle has an opening window and a rotating cutter. Tissue is vacuumed into the window as the rotating cutter advances forward to collect the sample. The cutter is withdrawn, and the vacuum system helps to transport the specimen to a tissue collection chamber (as shown in Fig. 22.4) Multiple specimens can be obtained with a single-needle insertion (range 6–18). The needle is positioned preferably under the target or, alternatively, near or inside; the vacuum mechanism produces a cavity around the biopsy site, so particular attention has to be taken for the skin, pectoral muscle, or vascular structures (Figs. 22.5 and 22.6).

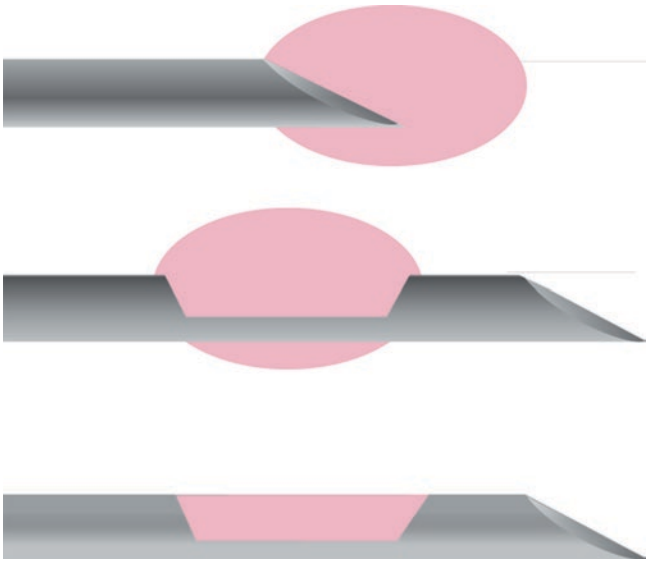

Fig. 22.2 CB needle: the needle is advanced until the tip is a few millimeters proximal to the edge of the lesion. The core biopsy gun is then fired for sampling

Vacuum-assisted devices provide larger-core samples than CB and enable more contiguous sampling potentially allowing more complete sampling of lesions, thereby lowering the chance of sampling error.

The larger-core specimen and contiguous sampling improve retrieval of calcifications and lower underestimation of cancer. In our experience, in a series of 406 cases, classified as BI-RADS 4, US-guided VAB procedure showed an accuracy of 100%, a sensibility of 97%, and a false-negative rate of 0.6% [9].

Complete removal of the imaging target is more likely after vacuum-assisted biopsy than after CB because of the larger volume of tissue removed. For these reasons VAB is preferable for calcifications, in cases where it is necessary to sample large amounts of tissue for lesions with complex histology, in case of discordance between imaging and cytological or histological examination (by CB), or if the cytology is inconclusive. Complete removal of the lesion does not ensure complete excision of the pathologic abnormality, especially for calcifications; thus, even in the case of complete removal of a malignant lesion, surgery is warranted.

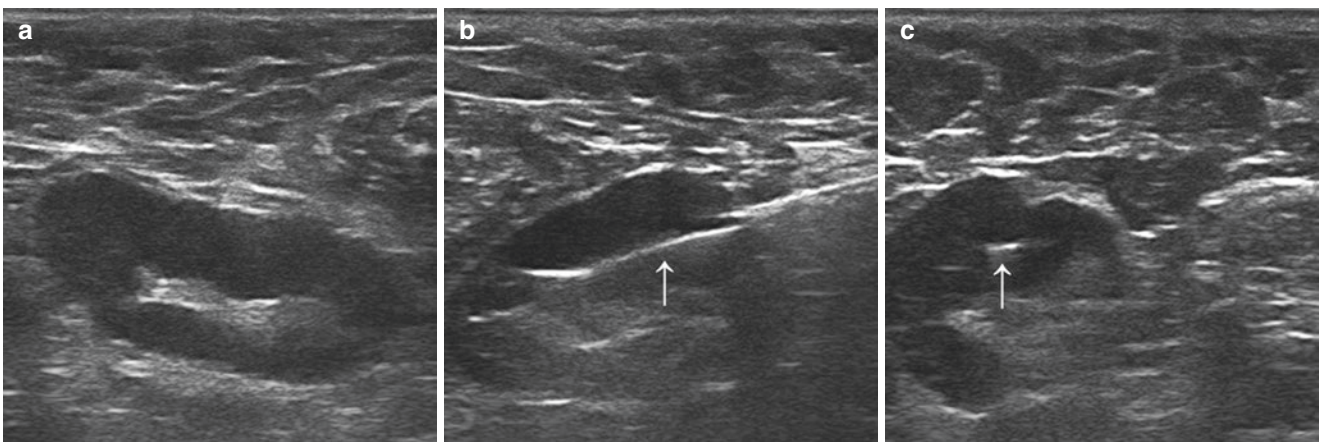

Fig. 22.3 (**a**) Core biopsy using a 14-G automated needle of a lymph node. (**b**) The needle is introduced into the lymph node along the direction of the long axis of the probe (longitudinal view). (**c**) The correct position of the needle is controlled rotating the probe of 90° (transversal view)

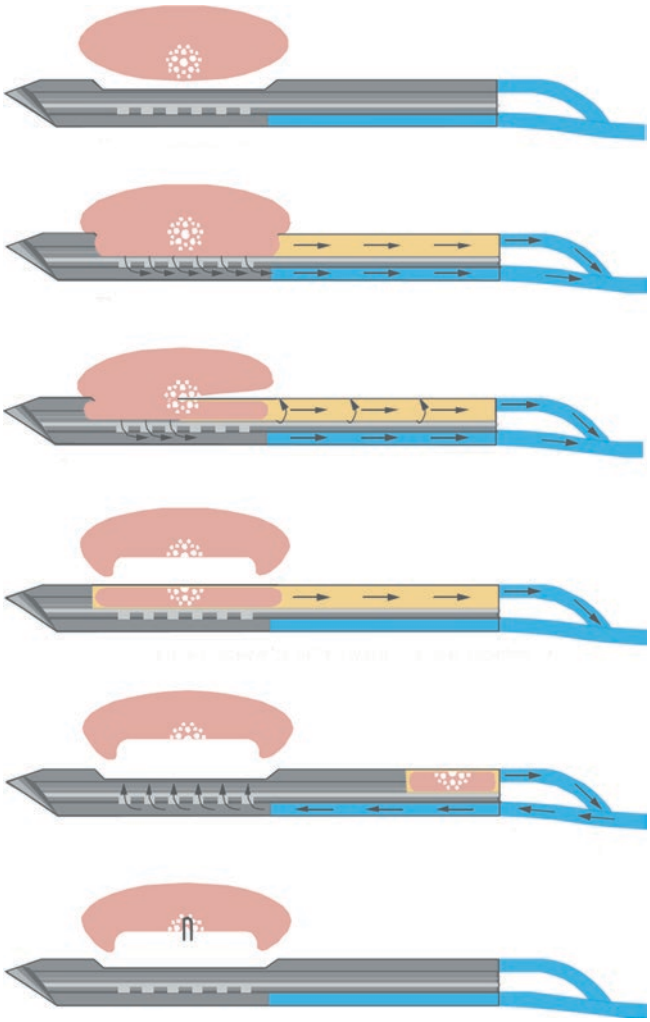

Fig. 22.4 Vacuum-assisted biopsy technique: the needle is placed under the target lesion and the tissue is vacuumed into the opening. The rotating cutter is advanced forward capturing a specimen that is transported to a collection chamber

Before considering any biopsy benefits, limitations and risks of the procedure as well as alternative procedures should be addressed to the patient, and a written informed consent has to be obtained. In the presence of coagulation disorders, anticoagulation drugs have to be stopped according to primary care physician or hematologist instruction. Some institutes recommend to discontinue therapy 1 week before the procedure, even herbal supplements such as ginkgo biloba, vitamin E, and fish oils.

Biopsy guided by the imaging is generally contraindicated if the patient is allergic to local anesthetics, if the undefined lesion is not visible or safely accessible, and if the patient is not cooperative. X-ray-guided biopsy should be avoided in pregnant women.

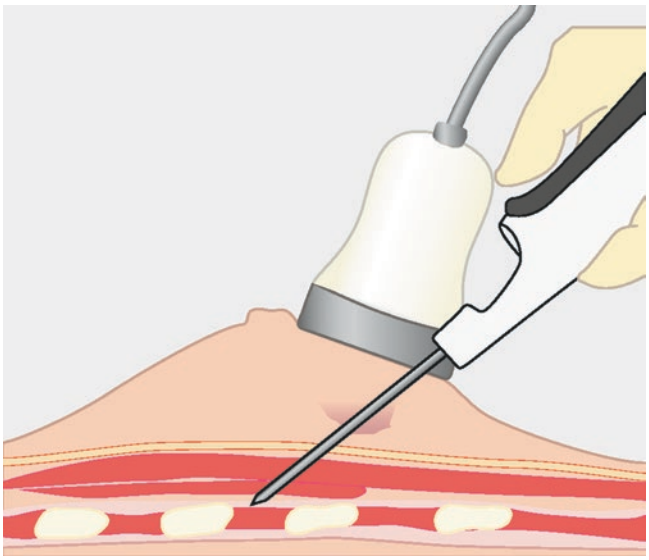

Fig. 22.5 Wrong VAB needle position: the needle is not parallel to the chest wall; there is risk of iatrogenic pneumothorax

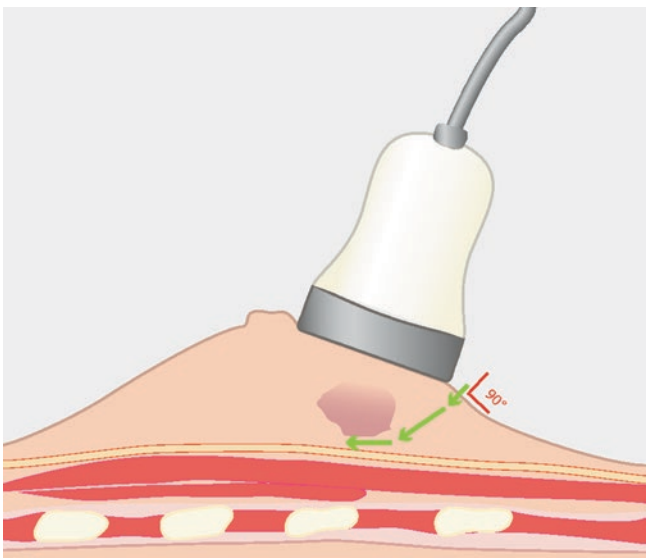

Fig. 22.6 Correct VAB needle position: the needle is placed parallel to the chest wall

22.3 Image Guidance Choice

Image guidance and type of needle choice are based primarily on target characteristics (imaging features, size and position), operator experience, and available devices (hospital resources). If a lesion is visible by more imaging tools, the radiologist has to choose the least invasive and easier access.

Ultrasound guidance, when feasible, is definitely the technique of first choice as it is easier to perform, more comfortable for the patient, and less time-consuming than the X-ray or MR-guided techniques.

If a target is evident in mammography and ultrasound, before initiating the biopsy, we must be assured that the ultrasound image matches the mammographic finding.

Ultrasound guidance can also be used (though not necessarily) in case of a palpable lesion to visualize the needle as it is advanced to the biopsy target. Ultrasound guidance can be used successfully also for smaller lesions or lesions that are located deep within the breast tissue or near to the nipple, regardless of the size of the breast or in the presence of prosthetic implants (Fig. 22.7).

During ultrasound-guided procedures, the patient is supine, and the radiologist selects the safest and most accurate way to proceed.

Stereotactic (X-ray) guidance is preferred for lesions identified by mammography but not adequately visualized by ultrasound. These lesions include microcalcifications, areas of parenchymal distortion/stellate lesions, or small soft tissue masses that are not visible on ultrasound.

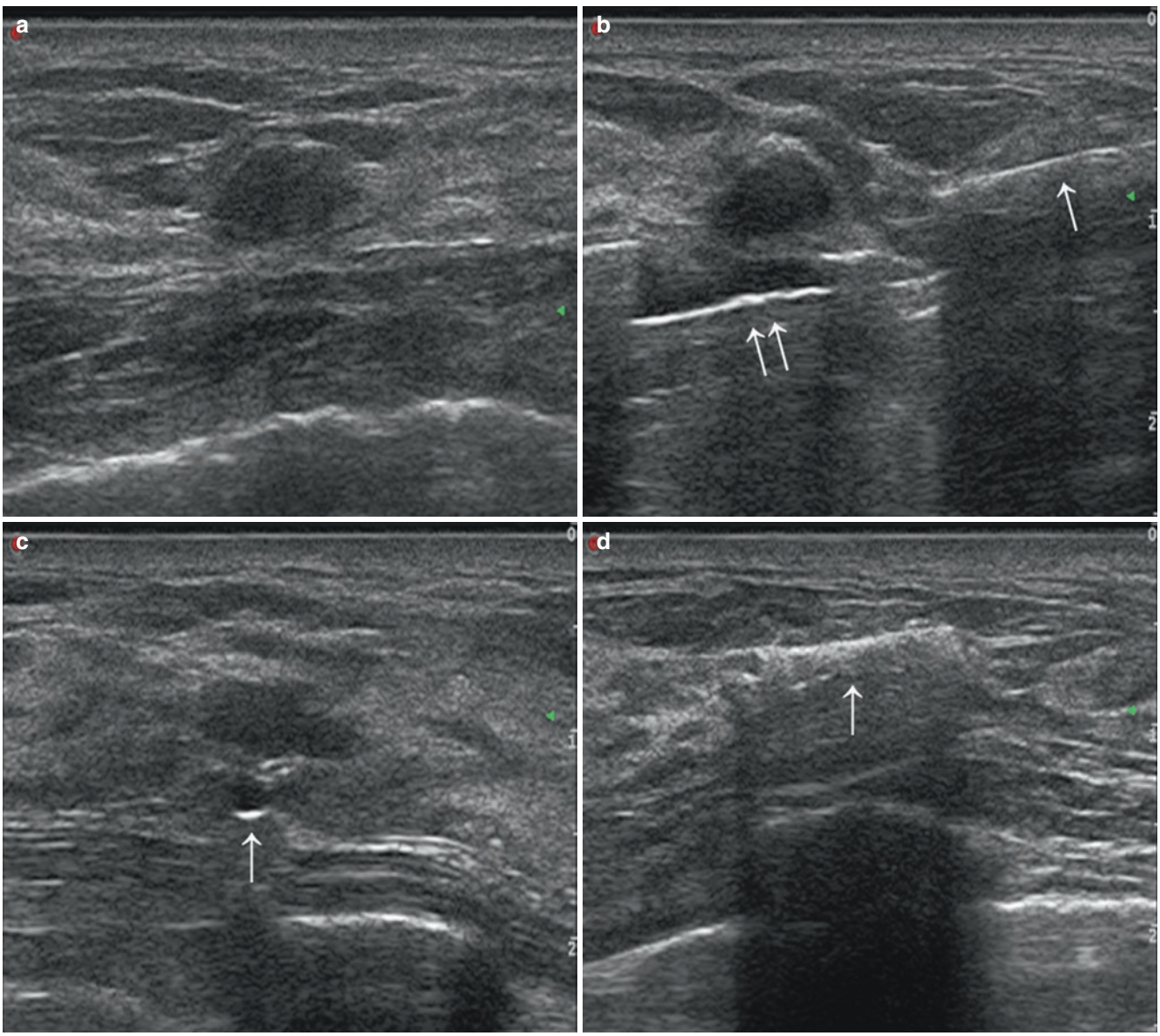

Fig. 22.7 (**a**) Ultrasound-guided VAB with 11-G needle of an ipoechoic lesion (dubious on FNA) in the right breast. Histology: fibroadenoma. (**b**) Longitudinal view: the needle is introduced under the lesion (*single arrow*), and the sampling window is open, facing the target in the center (*double arrows*). (**c**) Transverse view: the needle is well visible under the lesion (*arrow*). (**d**) The lesion is completely removed and a clip is placed (*arrow*)

The patient is usually positioned prone on a dedicated examination table (sometimes upright or in decubitus position, depending on the machine) with the breast hanging freely through an opening. A mammography unit is attached to the table and allows operator to identify the lesion to biopsy. The technician takes three scout radiographs (at 0° and two lateral views at –15° and +15°). The radiologist chooses two out of the three scouts to select the target and estimate exactly the depth of the lesion. After local anesthetic injection, the technician retakes the previously selected scout images to verify that the position of the target has not changed. The radiologist fires the needle into the breast and verifies post-fire scout images and sample specimens.

When sampling areas of microcalcifications, a radiography of the core samples is performed to ensure that tissue collected contains microcalcifications. If they are not present or are too few, the radiologist may decide to sample extra tissue (Fig. 22.8).

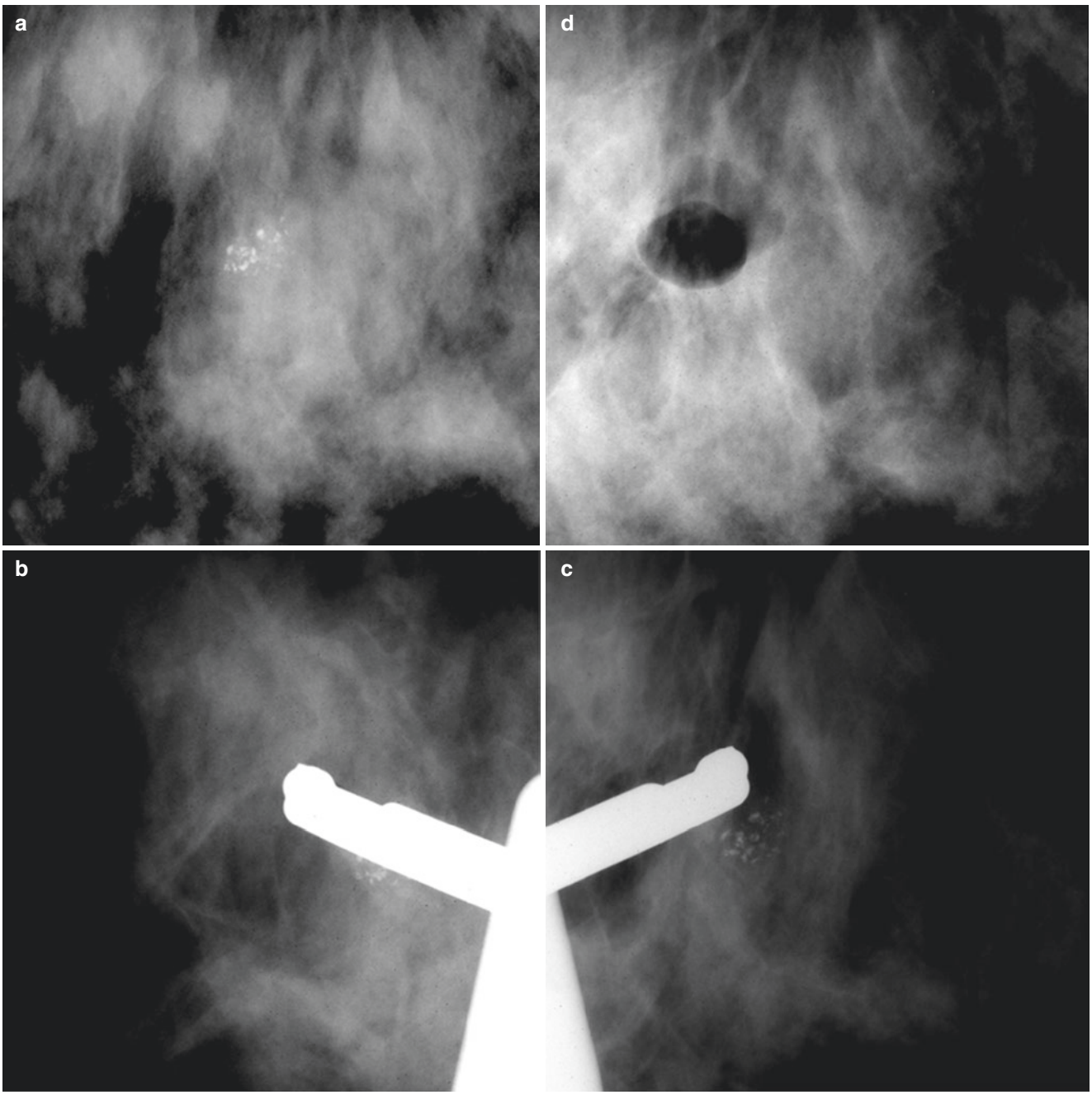

Fig. 22.8 (**a**) Stereotactic-guided VAB with 11G needle of clustered irregular microcalcifications. Histology: ductal carcinoma in situ. (**b**), (**c**) Post-fire views at –15° and +15° off perpendicular demonstrate correct needle position. (**d**) The target is completely removed and a radiolucent cavity is visible at the end of the procedure

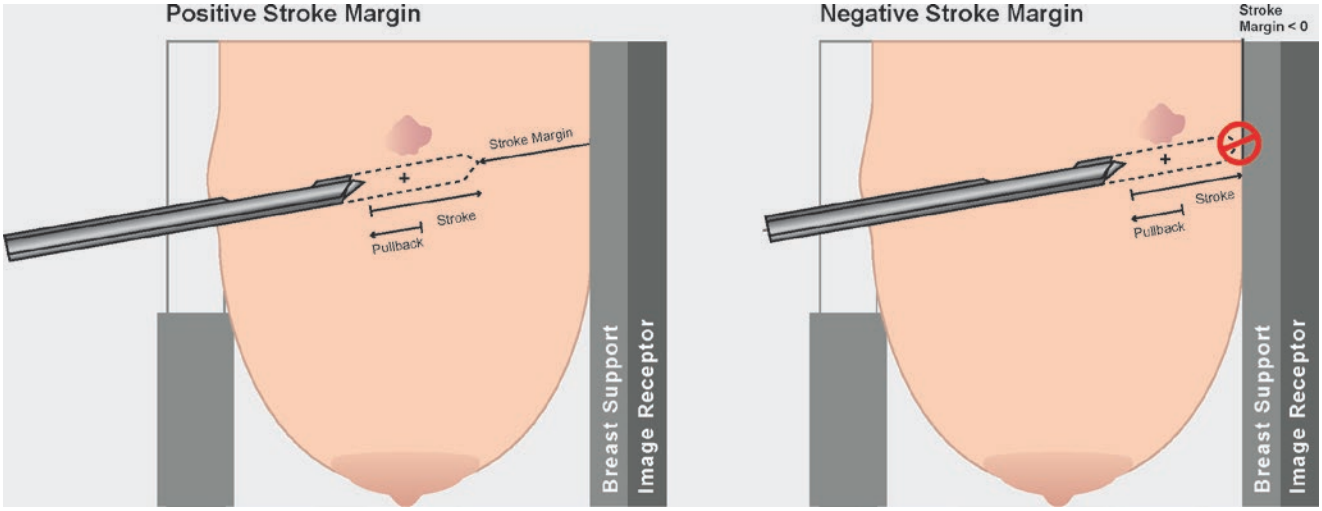

Fig. 22.9 Stroke margin is the distance between the post-fire needle position and the image receptor. Negative stroke margin indicates that the needle will exit the breast and strike the image receptor

Limitations of stereotactic biopsy are related to difficulty in reaching the target for small breast size which yields a negative stroke margin (distance between the post-fire needle position and the image receptor as shown in Fig. 22.9) or for inaccessible position (e.g., close to the nipple or chest wall or skin). In these cases surgical excision is necessary.

MRI guidance is indicated when the target is visible only by magnetic resonance. If the MR finding is visible on US or MX also on hindsight, then it is preferable to proceed with US-guided or MX-guided biopsy. It is important to understand the differences in breast positioning between the three imaging methods and the effect that this will have on lesion location.

MR-guided biopsies are performed with VAB device on only 1.5 or 3.0 T magnet, using a dedicated breast coil. MR-guided procedures are not yet widely diffused given they are more difficult to perform, longer in duration (30–60 min), and more expensive than the other techniques described.

Nevertheless, the large increase in MR use associated to the low specificity of this method has determined a significant growth of these biopsy procedures.

During the procedure the patient is in prone position with the breast compressed between the grid and an often-solid plate. Breast compression is modest since tight compression may cause masking of the enhancement.

T1 pre- and post-contrast images are acquired to select the target. MR does not allow real-time monitoring of needle advancement and biopsy. Instead, the MR table is drawn out from the magnet to place the needle after local anesthesia and then reintroduced into the magnet for each check, in a step-wise approach. Once adequate needle placement is confirmed, samples are taken outside of the magnet to avoid image distortion from the needle (Fig. 22.10).

The clip is always placed after the procedure. Limitations are related to unreachable target due to breast size or lesion position. Sometimes, on the day of biopsy, the target disappears especially in the case of non-mass-like lesions. This is due to different breast compression or to hormonal changes. It is mandatory to repeat the MR after short interval to confirm that the scan is negative.

If MR biopsy cannot be performed, MR can be used to center the lesion to guide surgical biopsy.

22.4 Clip/Marker Placement

At the end of any VAB procedure (sometimes even in CB), it is possible to place a magnetic marker to help localize biopsy site in case surgery is needed.

A variety of clips are available; they are generally composed of different metals, with a number of shapes all of which are visible on radiograph. Clips may be surrounded by cylindrical plugs of material (such as porcine gelatin, artificial polymers, and bovine collagen) that inhibit migration and facilitate subsequent ultrasound visibility for 2–6 weeks following placement.

In the case of lesion that has been completely removed bioptically, the marker is mandatory. In the presence of residual disease, it could be dismissed.

After a MR biopsy, the clip is always necessary because of the impossibility to understand real time whether or not the target has been completely removed (due to bleeding or air bubbles that can blur the biopsy site).

The correct marker placement is controlled by the radiologist real time on the monitor during US-guided procedures or on two orthogonal mammograms for stereotactic or MR-guided biopsies (Figs. 22.7 and 22.10).

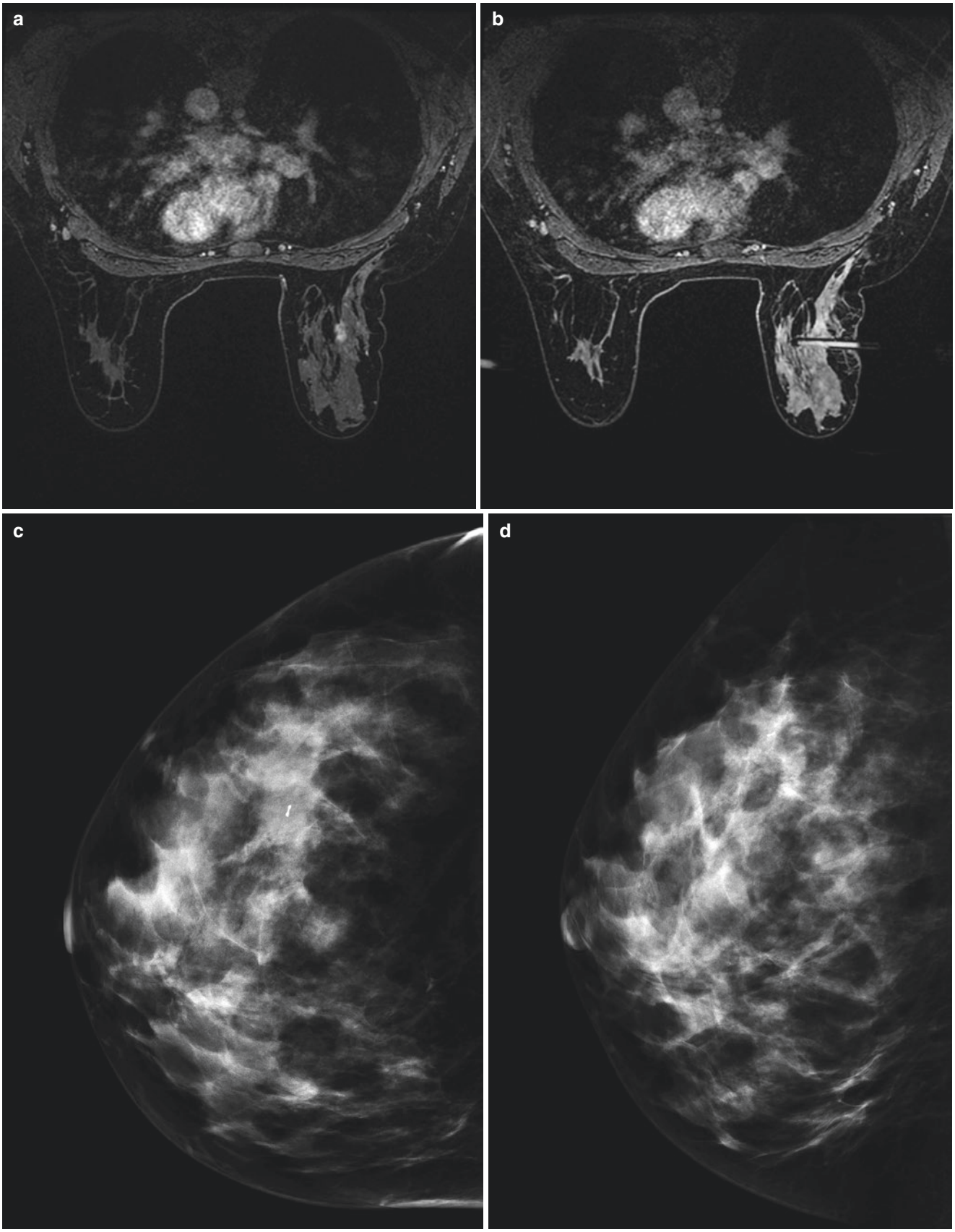

Fig. 22.10 MR-guided VAB with 8G needle of an irregularly shaped enhancing mass in the left breast. Histology: ductal intraepithelial neoplasia (DIN 3). (**a**) Axial contrast-enhanced subtraction image shows target lesion. (**b**) Contrast-enhanced subtraction image shows the biopsy needle in correct position. (**c**) and (**d**) two orthogonal post-biopsy mammograms show that the clip is in place

Post-needle biopsy errors in marker placement include:

- Non-deployment (rare and recognized the day of biopsy)
- Inaccurate initial deployment (common and recognized the day of biopsy)
- Delayed migration from the initial deployment site (rare and recognized days to months after biopsy)

If the marker has migrated from the biopsy site for more than 7 mm, another marker should be placed. In any case it is useful to describe the migration distance from the biopsy site in the medical report.

Alternatively, especially in Europe, carbon particles are sometimes used. These are injected along the needle trajectory to track a dark line in the breast, useful to guide surgery days to weeks after the biopsy.

22.5 Complications

The most frequent complication during and after a percutaneous biopsy is bleeding with consequent hematoma, rarely significant, which is treated with manual compression (up to 30 min), bandaging, and ice. Surgery is rarely necessary.

Less frequent complications include vasovagal reactions.

Very rare complications include infection, pneumothorax, pseudoaneurysm formation, implant rupture, and milk fistula (in pregnant or lactating women). Pneumothorax can occur when the target is very close to the pectoral muscle or rib cage, in noncompliant patients (moving or coughing), or if the needle angle is very steep.

Theoretically percutaneous biopsies can determine displacement of benign or malignant epithelium into tissue along the needle track. This is less frequent for vacuum-assisted biopsy than it is for fine needle aspiration or automated core biopsy (which requires multiple needle passes). There is no evidence that displaced cells are of biologic significance, but displaced epithelial cells can lead to difficulties in histologic interpretation by the pathologist (e.g., displaced DCIS can mimic infiltrating carcinoma). The pathologist should be aware of the findings of epithelial displacement to avoid misdiagnosing DCIS as infiltrating ductal carcinoma or multifocality in case of unifocal disease.

The available literature data does not document an increase in morbidity associated with iatrogenic seeding of the needle tract, although the certainty of the theoretical risk of local recurrence or metastatic spread cannot be definitely excluded. The continuous postsurgical follow-up accorded to patients, however, allows to control this risk [10–12].

At present, the patient benefit of preoperative biopsy diagnosis greatly outweighs the potential risks related with this procedure.

22.6 Management of Results

Every biopsy result has been submitted to concordance analysis.

Imaging-histologic concordance occurs when histologic results correlate with the imaging characteristics.

Imaging-histologic discordance occurs when histologic findings do not provide a sufficient explanation for the imaging features; this is most likely due to false-negative or (less frequently) false-positive results (in case of FNA). Imaging-histologic discordance may happen when BI-RADS category 4 or 5 lesions have benign histologic results or in case of targeting or sampling error.

Targeting error occurs when a sample from a lesion with associated microcalcifications has no calcifications identified by the pathologist or when a discrete mass lesion produces only normal breast tissue.

Sampling error occurs in lesions composed of inhomogeneous tissue when biopsy samples tissue that is not representative of the more altered portion of the lesion (e.g., sampling of only ADH in case of a DCIS associated with ADH).

Literature data reported that in the presence of imaging-histologic discordance after MR biopsies, malignancy rate ranges between 13 and 44% [13–16].

Repetition of biopsy, eventually using a larger needle or surgical biopsy, is recommended in case of imaging-histologic discordance.

Open biopsy procedures are not required in patients with histologically benign findings on percutaneous biopsy if imaging is concordant with the diagnosis. Instead, 6 or 12 or 24 until 36 months of follow-up is accepted (6 months of follow-up in case of MR-guided VAB).

If at follow-up a lesion increases in size or changes morphology or other features, it is necessary to repeat the biopsy or to surgically remove the lesion.

Surgical excision is imperative in case of malignancy, even in the presence of a DCIS after complete removal of the imaging target on biopsy. In our study conducted on 4.047 stereotactic VAB with 1594 cases of complete removal of the target lesion, the DCIS underestimation rate was low (5.5%) but not sufficient to consider VAB as a therapeutic procedure [17]. The underestimations risk in case of DCIS has been well documented and is related to lesion size (increased for lesions >20 mm), presence of residual tissue after biopsy, lesion type (mass lesion vs. microcalcification), biopsy device (CB vs. VAB), number of specimens obtained (≤10 vs. >10), and patient age [18, 19].

22.7 High-Risk Lesion Diagnosed at Breast Biopsy

The reported possibility to obtain a B3 lesion, defined also as high-risk lesion, after a percutaneous breast biopsy ranges between 3 and 10% [20–25]. Some authors consider these

lesions as "non-obligate precursors of malignancy." In other words, if these lesions are not removed, they have potential to become cancerous over time. Other authors consider them as "indicators of risk," which means that they increase the possibility of developing a breast cancer in the same or in the contralateral breast.

For these reasons management of B3 lesions is controversial. There is a large amount of literature data recommending surgical excision over follow-up [26, 27] because of the significant possibility of their upgrade to malignancy (B4–B5) when a larger amount of tissue is sampled [28–30] even in case of complete removal of the imaging target during biopsy [31].

In accordance with international debate, each case should be discussed in multidisciplinary meetings to assess biological risk, representative sampling, lesion size and extent, percentage of lesion removal, and other individual risk factors. The possibility of surveillance should be considered before selecting any management of a lesion.

22.7.1 Atypical Ductal Hyperplasia (ADH)

Atypical ductal hyperplasia is a complex lesion that histologically has some but not all microscopic features of (low-grade) DCIS. Usually, ADH is associated with or nearby suspicious microcalcifications (>80%) that are frequently the target of stereotactic breast biopsy. ADH can also present as a mass lesion without calcifications. Atypias may be also associated with radial scar or contained within fibroadenomas, papillomas, or non-tumorous benign lesions.

The downstaging risk is 20–56% with CB and 11–35% with VAB [5, 32–38]. In our experience using stereotactic VAB (11- and 8-G needles), the risk of downstaging ADH was not negligible (6.6%), even in case of complete target removal [17].

After MR imaging-guided biopsy, the reported risk of downstaging ADH is very significant, up to 50% [13, 39–42].

Due to its histological characteristics and significant number of underestimates to date, usually surgical consultation to discuss excisional biopsy is recommended [43].

22.7.2 Lobular Neoplasia (LN)

Lobular neoplasias include atypical lobular hyperplasia and lobular carcinoma in situ with classic morphology that, like ADH, may have a considerable spectrum of histological appearances without distinctive imaging findings. LNs on mammography typically present as microcalcifications; on ultrasound they present as mass lesions or aspecific architectural changes. LN can be found within fibroadenomas or within other benign lesions. Sometimes they are found incidentally on biopsy performed for other reasons or on surgical specimens.

Currently there is an open debate on the need to excise a breast target that yields LN as the highest-risk lesion at biopsy because of the significant underestimation rate reported after VAB (range 15–33%, 17% in our series) [44–47], making surgical consultation still recommended, especially for LCIS [48].

Nevertheless, in selected cases (patients not considered at high risk or without a past or present diagnosis of breast carcinoma), clinical management of LCIS and ALH can be an effective alternative to surgical excision when radiological and histological characteristics are well defined and suggest a low potential for an upgrade at the time of excision [49, 50].

22.7.3 Papillary Lesions (PL)

Papillary lesions are a heterogeneous group that includes benign intraductal solitary papillomas, atypical papillomas, and multiple papillomas. For benign papilloma the upstaging risk is reported to range from 0 to 7% [51, 52]. Generally, in case of complete VAB removal, conservative treatment should be strongly considered except in those patients with papilloma associated with mass lesions or with imaging/histologic discordance. Data reported that in atypical papillomas, there is a risk of malignancy of up to 67%, making surgical excision necessary [53]. Multiple breast papillomas are characterized by numerous papillomas causing mass-like lesions associated with complex sclerosing lesions and atypical hyperplasia, generally located in the periphery of the breast. Because of their associated risk of malignancy, lesions larger than 1.5 cm should be surgically removed [54, 55].

22.7.4 Radial Scar and Complex Sclerosing Lesion

Radial scars are tumorlike lesions with architecturally distorted glandular tissue that may be difficult to differentiate from low-grade carcinoma in histological section [56]. Complex sclerosing lesions are radial scars greater than 1 cm in size. At mammography they appear as architectural distortions while at ultrasound, if visible, like an ipoechoic irregular mass with posterior shadowing [57]. The upstage risk ranges from 0 to 22% [54].

Surgery is recommended if cellular atypia is present. In cases without atypia and complete removal with VAB, short-term follow-up is appropriate [58].

22.7.5 Phyllodes Tumors

Phyllodes tumors show as masses ranging from 1 to 10 cm, with circumscribed margins that may appear indistinct at

mammography. They can be benign, borderline, or malignant (23–50%) [5]. Surgical excision is always warranted even for benign tumors which can still be locally aggressive.

22.8 Alternative Biopsy Methods

The goal of percutaneous breast biopsy is diagnosis, not treatment; however, in specific cases, this concept is changing.

In the USA an innovative biopsy method that captures breast tissue for histological analysis using RF (radiofrequency) energy, defined as BLES system (breast lesion excision system (BLES)), has been introduced since 2001. The BLES consists of a biopsy "wand" that is placed (after local anesthesia and skin incision) at the edge of the target under ultrasound or stereotactic guidance. Five metallic prongs (depending on wand size), with their tips connected by an extensible cutting radio-frequency ring wire, pass from the wand and envelop an area of tissue ranging from 10 to 20 mm in diameter (depending on wand size) in only 8 s (Fig. 22.11) [59]. The prongs pass RF waves into surrounding tissue in order to excise and allow hemostasis, but not to the extent of damaging the sample. The stereotactic guidance seems to be more accurate because during the procedure the breast is maintained in a static position. Compared with VAB, this procedure can export a single entire sample keeping the architecture intact and margins clear. This facilitates the work of the pathologist and reduces the risk of underestimation (especially for histologically complex lesions) [60]. Furthermore, it may allow the removal of small borderline or benign lesions, thus eliminating the traditional wide local surgery [60–62]. The procedure is well tolerated with few complications. It is contraindicated in patients with a cardiac pacemaker or other radio-frequency devices and in patients who are pregnant. Caution is recommended in patients taking anticoagulation drugs and in patients with clotting disorders. During the biopsy the RF waves emanating from the metallic prongs produce heat that could burn the skin; for these reasons it cannot be used in lesions too close to the skin or to the chest wall, in small breasts, or in the axilla [59, 63].

Other recent ultrasound technical developments include three-dimensional (3D) US-guided biopsy and biopsy conducted with tissue harmonic imaging (THI) and compound imaging (CI).

The 3D-guided biopsy allows a better understanding of the position of the breast lesion and the surrounding tissue, without needing to change the transducer plan, especially in small lesions. The precise point of post-fire is visualized in a multiplanar display, facilitating the procedure. So far the

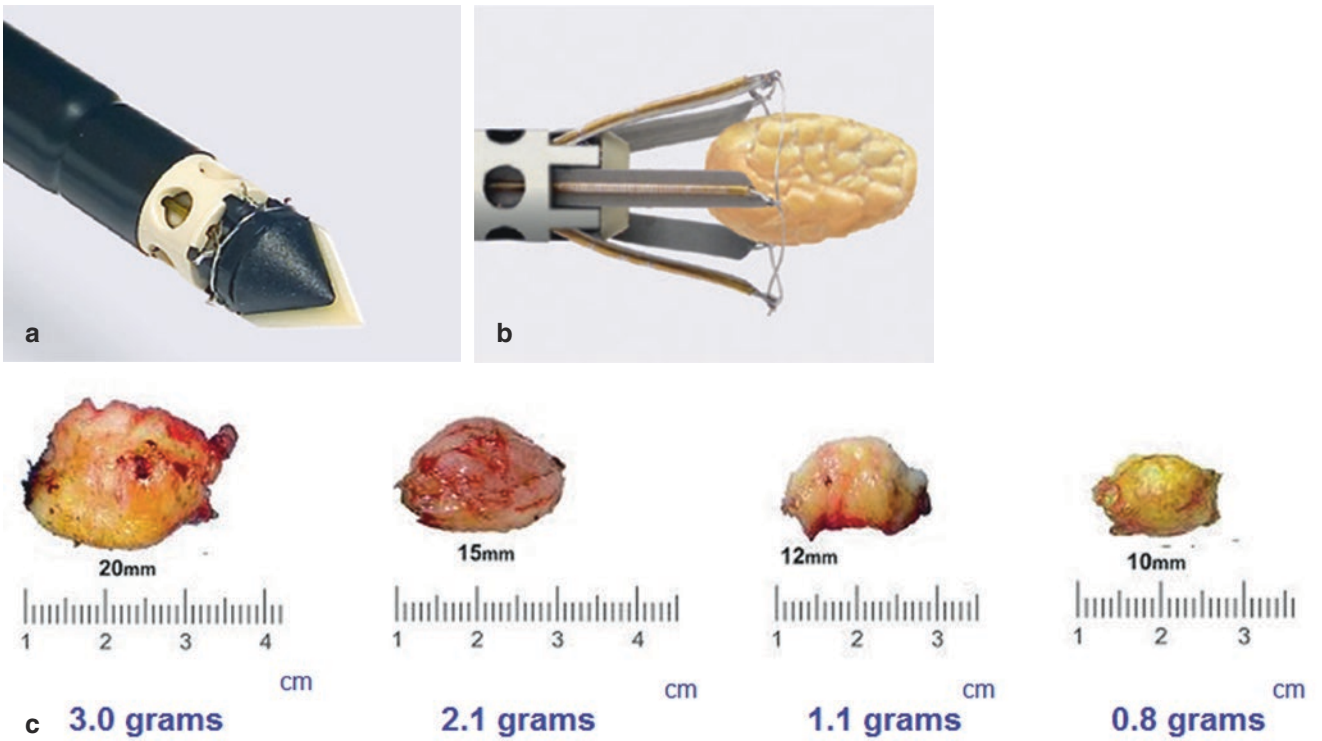

Fig. 22.11 (**a**), (**b**) BLES capture basket: five small RF-enabled wires exit from the wand to circumscribe the lesion. As they proceed, they draw out five supporting elements which support and cradle the sample for withdrawal. (**c**) Specimen samples are available in four sizes: 10-, 12-, 15-, and 20-mm diameters

reported rate of false negatives and underestimation is similar to that of the 2D system [64, 65]. It is a safe procedure and appears to be more useful for nonexpert in freehand positioning operators or in centers where the number of requested breast biopsies is small [66]. More studies are needed to define the real value of this method.

Tissue harmonic imaging (THI) and compound imaging (CI) were applied to ultrasound machine to enhance the detectability of the biopsy target, especially when the surrounding tissue appears as hypoechoic as the target lesion. THI creates images generated by US-tissue interactions solely from higher frequencies. This enhances tissue contrast between the lesion and the surrounding tissue [67]. THI can be useful in the visualization of a lesion surrounded by fatty background, but is of limited value for needle visualization during biopsy [68].

Compared with the B mode imaging, the single image obtained with the CI has more defined margins and internal architecture because it is created from multiple frames and from different frequencies (frequency CI) or from different angles (spatial CI) [68, 69]. Therefore CI improves the visibility of the lesion and the needle in breasts with a glandular background and suppresses lesion shadowing.

Despite these apparent advantages, conventional B mode is still considered the best compromise in breast biopsy.

In recent years, technological innovations have included also the tomosynthesis with digital mammography. Tomosynthesis acquires, with a low dose of radiation and in a short period of time, multiple images to predefined levels throughout the breast volume. It helps identify benign or suspicious masses in dense breasts, usually not visualized with conventional digital mammography, and gives information on their exact depth and location. Generally, in the presence of a suspicious lesion highlighted by tomosynthesis, without corresponding ultrasound image, the patient is subjected to an MR exam and to a possible subsequent MR-guided biopsy. However, targets visualized only on 3D mammography can be biopsied using tomosynthesis-guided VAB. Further studies are needed to demonstrate the clinical significance of this innovation [70–72].

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Part V

Oncological Surgery

Conservative Surgery

23

Umberto Veronesi

23.1 Premises

Surgical oncology showed a great evolution over the past century. The increased incidence of malignancies leads to an increased interest in discovering new diagnostic and therapeutic techniques. Since the end of the nineteenth century, the objective of the surgeons was to offer the "maximal tolerable treatment," as the effort was concentrated on delivering as extensive surgery as could be tolerated, in order to improve disease control. This approach resulted in devastating interventions with a negative effect on the quality of life. At the end of the sixties, when it was made clear that the prognosis was mainly linked to the presence or absence of distant metastases and not to the extent of local treatment, the opposite trend was developed with the objective to identify the "minimal effective treatment," aiming at preservation of the affected organ, that would improve the patients quality of life.

The advances in the field of systemic treatments led to an improved control of the disseminated disease. Adjuvant chemotherapy and endocrine therapy became therefore component of the treatment. More recently the progress in the knowledge on cell genetic is leading to targeted treatments increasing the therapeutic efficiency and sparing the patient the side effects of chemotherapy.

Radiotherapy is often essential for obtaining a good local control. The progression of informatics technology changed the radiation treatment from two-dimensional radiotherapy to three-D conformal radiotherapy and recently to intensity-modulated radiation therapy.

Since the publication of the paper on a study conducted from January 1988 to December 1989 [1], neoadjuvant treatments are being used for several oncologic patients for downstaging. Chemotherapy, as well as hormone therapy for hormone-dependent tumors, in the preoperative setting is shown often to decrease the extent of the solid tumors, rendering them operable, often with the conservation of the organ.

23.2 Value of Randomized Trials

Randomized trials have contributed significantly to the changes in surgical oncology in breast cancer that were fundamental for all the revolutionary changes performed. An attempt to improve the prognosis through a more extended treatment was the aim of the trial on internal mammary node dissection. A large international randomized trial was published in 1976 comparing radical mastectomy with or without internal mammary dissection [2]. From 1963 to 1968, 1,453 patients in five centers were randomized. The 5-year survival was similar in the two groups. The Cancer Institute of Milan participated in this study and published the 10-year follow-up of 716 patients in 1981 [3], without differences in overall survival and disease-free survival in the two groups. There was no difference in recurrence rates on the operating field, the axilla and the supraclavicular fossa. The 10-year update of the multicenter study that was published 2 years later confirmed no difference in survival and in relapse-free survival [4]. This first large trial attempted to explore the impact of more aggressive surgery failed the goal and inspired myself to look at opposite solutions. The passage from "maximally tolerated" to "minimally effective" treatment has not been easy, and the idea of conserving a large portion of an affected organ was challenged by many surgeons and medical oncologists. It was only the large randomized trials performed in breast cancer patients that made possible the acceptance of breast conservation and led to a complete modification of the principles of breast surgery.

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23.3 Breast-Conserving Surgery

Over the years, Halsted mastectomy has been replaced by lumpectomy or quadrantectomy, with external high-energy radiotherapy as an integrated component of treatment (Figs. 23.1 and 23.2).

A milestone was the publication in 1981 of the results of a randomized trial that compared Halsted mastectomy with breast-conserving surgery plus complete axillary dissection plus full-dose radiotherapy to the breast [5]. The trial, which recruited 701 patients with tumor ≤2 cm, showed no difference in survival between the two groups. The findings of the Milan trial were confirmed by long-term follow-up published in 1981 [6] (Figs. 23.3 and 23.4).

In the USA, Fisher and colleagues, 5 years later, adopted a slightly more conservative approach. Their trial, published in 1985 [7], compared a more limited tumor resection (initially defined partial mastectomy and later lumpectomy) with a total mastectomy that included removal of the fascia overlying the muscles but not the muscles themselves. As in the Milan trials, patients with stage I–II breast cancer were eligible, but maximum tumor diameter was 4 cm. Patients were treated with lumpectomy only if resection margins were negative. Axillary dissection was generally more limited than in the Milan trial.

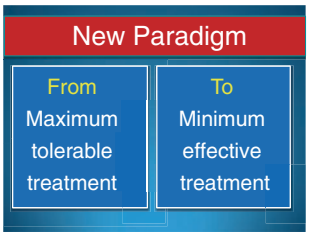

Fig. 23.1 Cancer surgery revolution from 1970

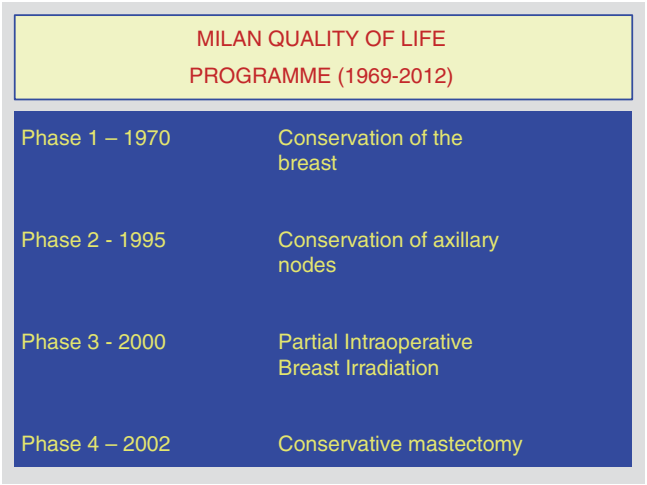

Fig. 23.2 Milan program for quality of life for breast cancer patients

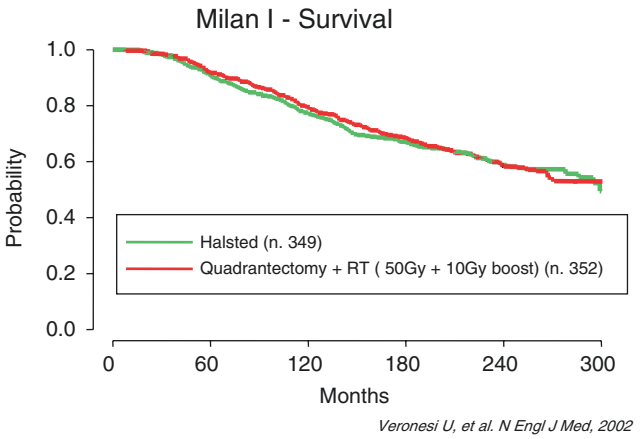

Fig. 23.3 Long-term survival of patients treated with Halsted mastectomy (349 patients) and quadrantectomy plus radiotherapy (352 patients)

Fisher found that distant disease-free survival and overall survival were no worse in the lumpectomy arm than mastectomy arm. The results were confirmed after a long-term follow-up [8].

In 1979, the National Cancer Institute conducted a prospective randomized study comparing modified radical mastectomy vs. lumpectomy—with resection margins, either positive or negative, with axillary dissection and adjuvant radiotherapy [9]. After 20 years of follow-up of 237 patients, OS and DFS were comparable; however according to the authors, "breast failures continued to occur throughout the follow up" [10].

A study with a similar design was launched in 1980 by EORTC. The trial randomized 868 patients with T1 and T2 tumors until 1986 to either modified mastectomy or lumpectomy—with positive or negative resection margins—with axillary dissection and adjuvant radiotherapy [11]. At 10 years, the results were similar to those of the NCI trial. Overall survival and distant metastasis-free survival were similar; however local recurrences were higher in the lumpectomy group.

Between 1983 and 1989, the Danish Breast Cancer Cooperative Group after randomizing 905 patients to either modified radical mastectomy or lumpectomy with axillary dissection and radiotherapy concluded that OS and DFS did not differ significantly [12].

These large randomized trials conducted in the 70s and early 80s showed the way to "less surgery" and practically changed the principles of breast cancer surgery. Furthermore, they confirmed the hypothesis that the prognosis of breast cancer patients is linked to the presence or absence of distant metastasis and changes in local treatment do not affect the overall survival. Breast conservation became a standard treatment, and the updates published at the beginning of the twenty-first century confirmed that mutilating interventions such as Halsted radical mastec-

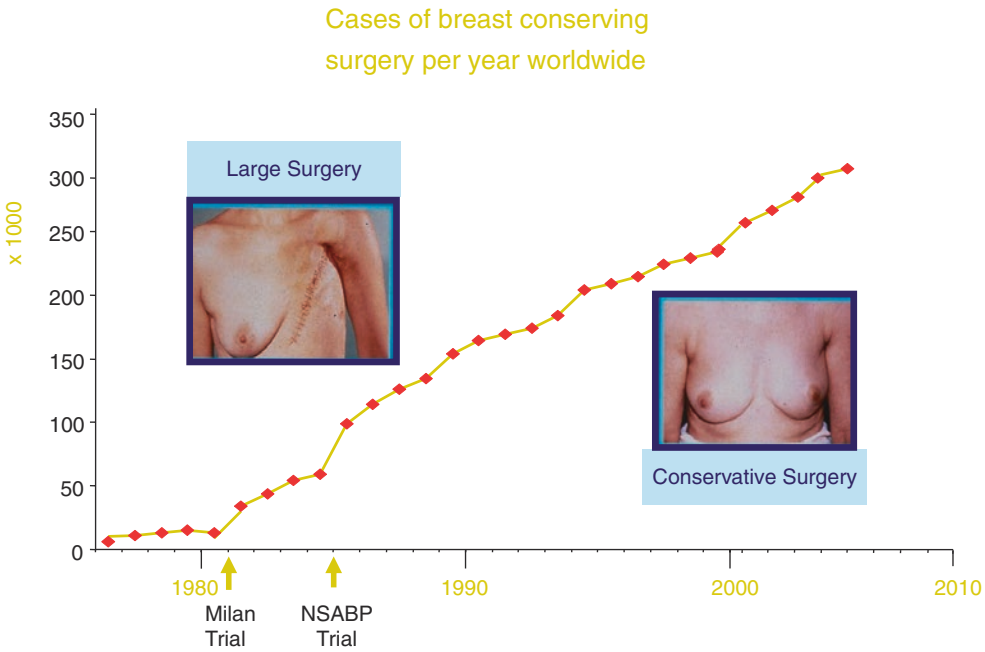

Fig. 23.4 Rough estimate of the increase of conservation of the breast worldwide

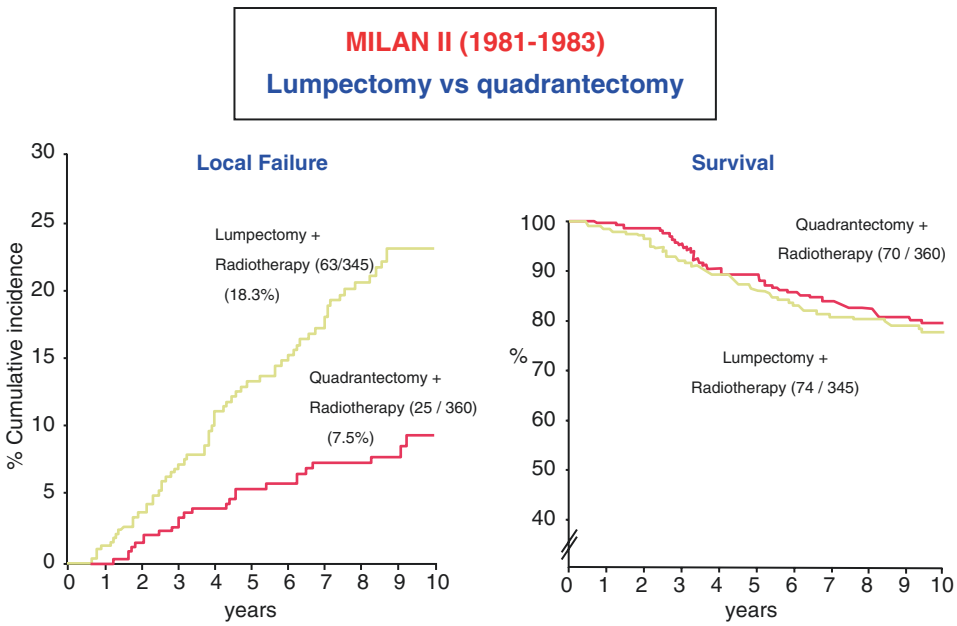

Fig. 23.5 Ten-year results of the randomized trial (705 cases) comparing lumpectomy vs. quadrantectomy (*left* Local Failure, *right* Survival)

tomy belong to the past. However, some uncertainty remained about the extent of the breast conservation. This issue was further investigated with a randomized study (Milan II) that was conducted between 1985 and 1987, and its results were published in 1990 [13]. Seven hundred and five patients with tumors up to 2.5 cm were randomized to receive either quadrantectomy or lumpectomy. All patients underwent axillary dissection and radiotherapy. In quadrantectomy, 2–3 cm of normal tissue surrounding the tumor was excised, as well as the tumor overlying the skin and the underlying fascia. In lumpectomy, only a rim of 1 cm around the tumor was excised. After a follow-up of 10 years, OS and distant metastasis rate were not different, while in breast tumor recurrence was significantly higher in the lumpectomy group [14] (Fig. 23.5).

23.4 Postsurgical Radiotherapy

Following the establishment of breast conservation as treatment of choice for early breast cancer, the role of radiotherapy on locoregional control remained to be clarified. The effects of adjuvant radiotherapy were evaluated by two randomized trials. The first was conducted at the Milan Cancer

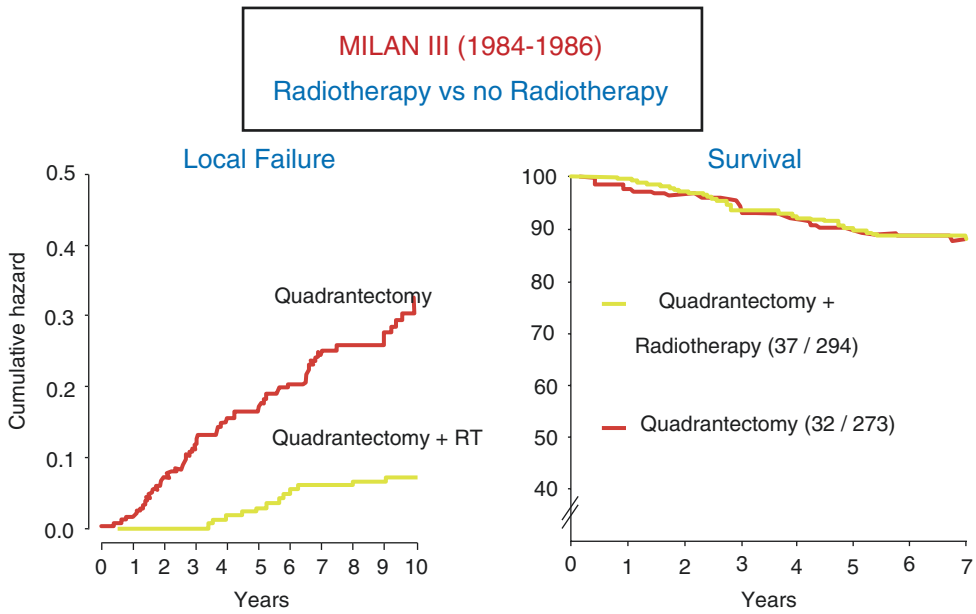

Fig. 23.6 Ten-year results of the randomized trail (567 patients) comparing postoperative radiotherapy vs. no radiotherapy (*left* Local Failure, *right* Survival)

Institute (Milan III) between 1987 and 1989 and recruited 567 patients with tumors up to 2.5 cm [15, 16]. They were randomized to quadrantectomy with axillary dissection with or without adjuvant radiotherapy. The radiotherapy group had a significantly lower local recurrence rate; however the 5-year overall survival was comparable. Similarly, the Uppsala-Orbero Breast Cancer Study Group reported the same conclusions in a study of 381 patients with pT1 tumors [17]. Radiotherapy is considered an important component of breast conservation, at least in women who are younger than 60 years old. For patients over 60 years old, a multicenter randomized trial was conducted, in order to assess the necessity of radiotherapy. Between 2001 and 2005, 749 patients with early breast cancer were assigned to either surgery only or to surgery and breast radiotherapy, and after 5 years of follow-up, a difference in breast recurrence (2.5% vs. 0.7%) was found, but no difference in overall survival and in distant disease-free survival [18] (Fig. 23.6).

23.5 Intraoperative Radiotherapy

Up to 85% local recurrences after conservative treatment develop in the scar area. This finding suggests that in many patients, only the tumor bed needs to be irradiated [18]. Furthermore, if this partial-breast irradiation could be given in single session and was noninferior to conventionally fractionated whole-breast irradiation, it would substantially ease the difficulties of women who have to contend with long waiting lists for radiotherapy or who live distant from a radiotherapy center. Such treatment would also be simpler and less expensive than conventional whole-breast irradiation. For these reasons, the European Institute of Oncology developed an intraoperative radiotherapy ELIOT (electron intraoperative therapy) technique that can deliver full-dose irradiation (21 Gy) over a few minutes during the surgery. The method employs a mobile linear accelerator that delivers an electron beam via an arm to which is attached a sterile cylindrical applicator. After cancer removal, the surgeon detaches the residual breast from the underlying fascia and inserts an aluminum-lead disk between the fascia and the gland to protect deep structures. The breast is temporarily reconstructed and the skin retracted out of the way. The energy of the electron beam (variable from 3 to 12 MeV) is selected based on gland thickness as measured by a needle [19–21].

In the year 2000, a randomized study was started at the European Institute of Oncology (Milan, Italy). Women aged 48–75 years with early breast cancer, a maximum tumor diameter of up to 2.5 cm, were assigned in a 1:1 ratio to receive either whole-breast external radiotherapy or intraoperative radiotherapy with electrons (ELIOT). Study coordinators, clinicians, and patients were aware of the assignment. Patients in the intraoperative radiotherapy group received one dose of 21 Gy to the tumor bed during surgery. Those in the external radiotherapy group received 50 Gy in 25 fractions of 2 Gy, followed by a boost of 10 Gy in five fractions. This was an equivalence trial; the prespecified equivalence margin was local recurrence of 7.5% in the intraoperative radiotherapy group.

One thousand three hundred five patients were randomized (654 to external radiotherapy and 651 to intraoperative radiotherapy) between November 20, 2000, and December 27, 2007. After a medium follow-up of 5–8 years (IQ 4.1–7.7), 35 patients in the intraoperative radiotherapy group and four patients in the external radiotherapy group had an IBTR ($p < 0.0001$). The 5-year event rate for IBRT was 4.4% (95% CI 2.7–6.1) in the intraoperative radiotherapy group and

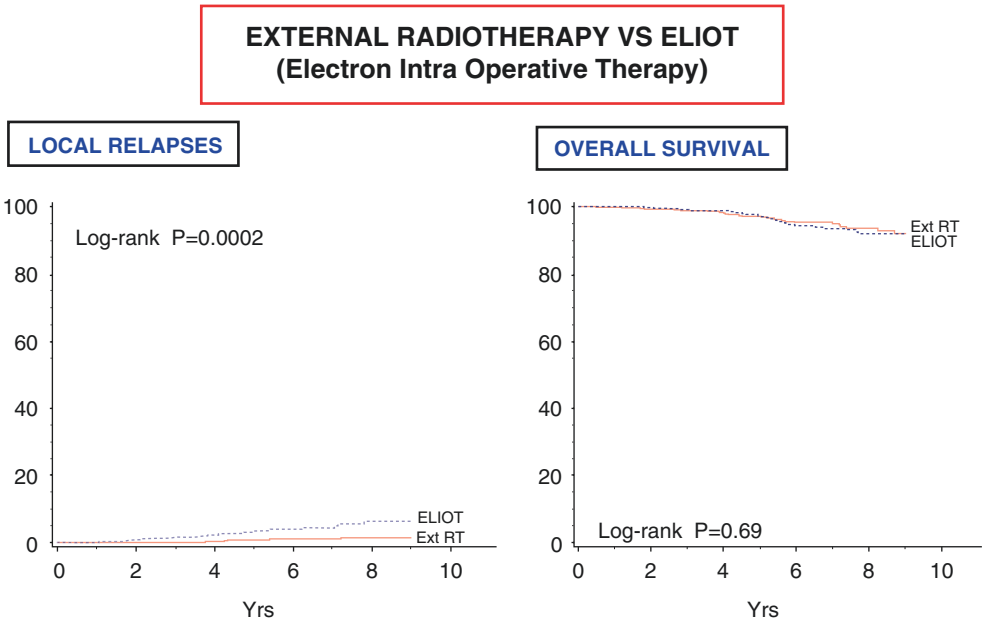

Fig. 23.7 Ten-year results of the randomized trial comparing external radiotherapy vs. intraoperative radiotherapy with electrons (1305 cases)

0.4% (0.0–1.0) in the external radiotherapy group (hazard ratio 9.3 [95% CI 3.3–26.3]). During the same period, 34 women allocated to intraoperative radiotherapy and 31 to external radiotherapy died ($p = 0.59$). Five-year overall survival was 96.8 (95% CI 95.3–98.3) in the intraoperative radiotherapy group and 96.9% (95.5–98.3) in the external radiotherapy group. In patients with data available ($n = 464$ for intraoperative radiotherapy; $n = 412$ for external radiotherapy), we noted significantly fewer skin side effects in women in the intraoperative radiotherapy group than those in the external radiotherapy group ($p = 0.0002$) [22].

In conclusion, although the rate of IBTR in the intraoperative radiotherapy group was within the prespecified equivalence margin, the rate was significantly greater that with external radiotherapy, and overall survival did not differ between groups. Improved selection of patients could reduce the rate of IBTR with intraoperative radiotherapy with electrons (Fig. 23.7).

23.6 Conservation of Axillary Nodes

The concept of "less surgery" was extended to the treatment of the axilla. The role of radiotherapy on the axilla was evaluated in a study conducted in Milan between 1995 and 1998 [23]. Four hundred and thirty five patients with small tumors, ≤1.2 cm, were randomized to either axillary radiotherapy or nothing. After 63 months of follow-up, the axillary metastases presented were lower than expected in both groups, suggesting that axillary dissection can be avoided in this subgroup of patients and that radiotherapy has a protective effect.

The introduction of the sentinel lymph node biopsy puts under investigation the role of axillary dissection. It was already anticipated that the positivity of the axilla was an element of prognosis and not a reason to perform more extensive surgery. Sentinel lymph node biopsy is a method of "predicting" the axillary status sparing the patient from axillary dissection and its often devastating complications, like arm lymphedema. As soon as the technique of sentinel lymph node biopsy was standardized, a series of randomized control studies started worldwide. The first was the Milan Trial that in 1998 and 1999 randomized 506 patients with tumors up to 2 cm to two arms, one receiving immediate axillary dissection and the other receiving the dissection only if the sentinel node was involved [24]. After 79 months of follow-up, OS and DFS were equal. Only one case of axillary recurrence was observed among the patients in the group who did not receive axillary dissection. The long-term analysis showed that patients had less mortality rates after sentinel lymph node biopsy policy than after immediate dissection (18 vs. 25 deaths).

An identical study was conducted between 1999 and 2004 that randomized 5,611 women with invasive breast cancer up to 4 cm from 80 centers in the USA and in Canada to either axillary dissection or to sentinel lymph node biopsy alone with axillary dissection only if the SLN was positive [25, 26]. After 95.6 months of follow-up, OS and DFS were similar in the two groups. A sub-study reported that up to 12 months postoperatively, patients with axillary dissection had significantly higher arm morbidity and significantly more restricted social activity and impaired QoL.

A multicenter UK trial, ALMANAC trial, studied the QoL in patients with SLN vs. axillary dissection between 1999 and 2003 [27]. One thousand and thirty one patients participated, and at 12 months, it was evident that lymphedema was higher in the axillary dissection group; operative time, drainage use, and hospitalization were much longer in

axillary dissection group, while in SLN group, patients had better arm functioning score. The results of the Danish Breast Cancer Cooperative Group confirmed the ones of ALMANAC. Arm lymphedema and dysfunction were significantly higher in the axillary dissection group at 12 months for ALMANAC and at 18 months for DBCCG [28].

It appeared clear that in case of absence of metastatic nodes, axillary dissection is not only unnecessary but also harmful. But what if the axillary lymph nodes are positive? Is axillary dissection still necessary or can it be avoided? The answer to this question is nowadays under investigation. The NSABP Z0011 trial has randomized 891 patients with T1 and T2 tumors and positive SLN from 115 centers from 1999 to 2004 to receive axillary dissection or no further treatment [29]. At 6.3 years of follow-up, the 5-year OS and the DFS were not different in the two groups, suggesting that prophylactic axillary dissection may not be necessary. The EORTC AMAROS trial has randomized patients with positive SLN to either axillary dissection or axillary radiotherapy from 2001 to 2010 [30]. At the European Institute of Oncology, a multicenter randomized, non-inferiority, phase 3 trial was conducted on 465 patients who had clinically nonpalpable axillary lymph node(s) and a primary tumor of 5 cm or less and who, after sentinel-node biopsy, had one or more micrometastatic (≤2 mm) sentinel lymph nodes with no extracapsular extension. Patients were randomly assigned (in a 1:1 ratio) to either undergo axillary dissection or not to undergo axillary dissection. Between April 1, 2001, and February 28, 2010, 465 patients were randomly assigned to axillary dissection and 469 to no axillary dissection. After a median follow-up of 5–0 (IQR 3.6–7.3) years, we recorded 69 disease-free survival events in the axillary dissection group and 55 events in the no axillary dissection group. Breast cancer-related events were recorded in 48 patients in the axillary dissection group and 47 in the no axillary dissection group [31] (Fig. 23.8). Another multicentric randomized trial

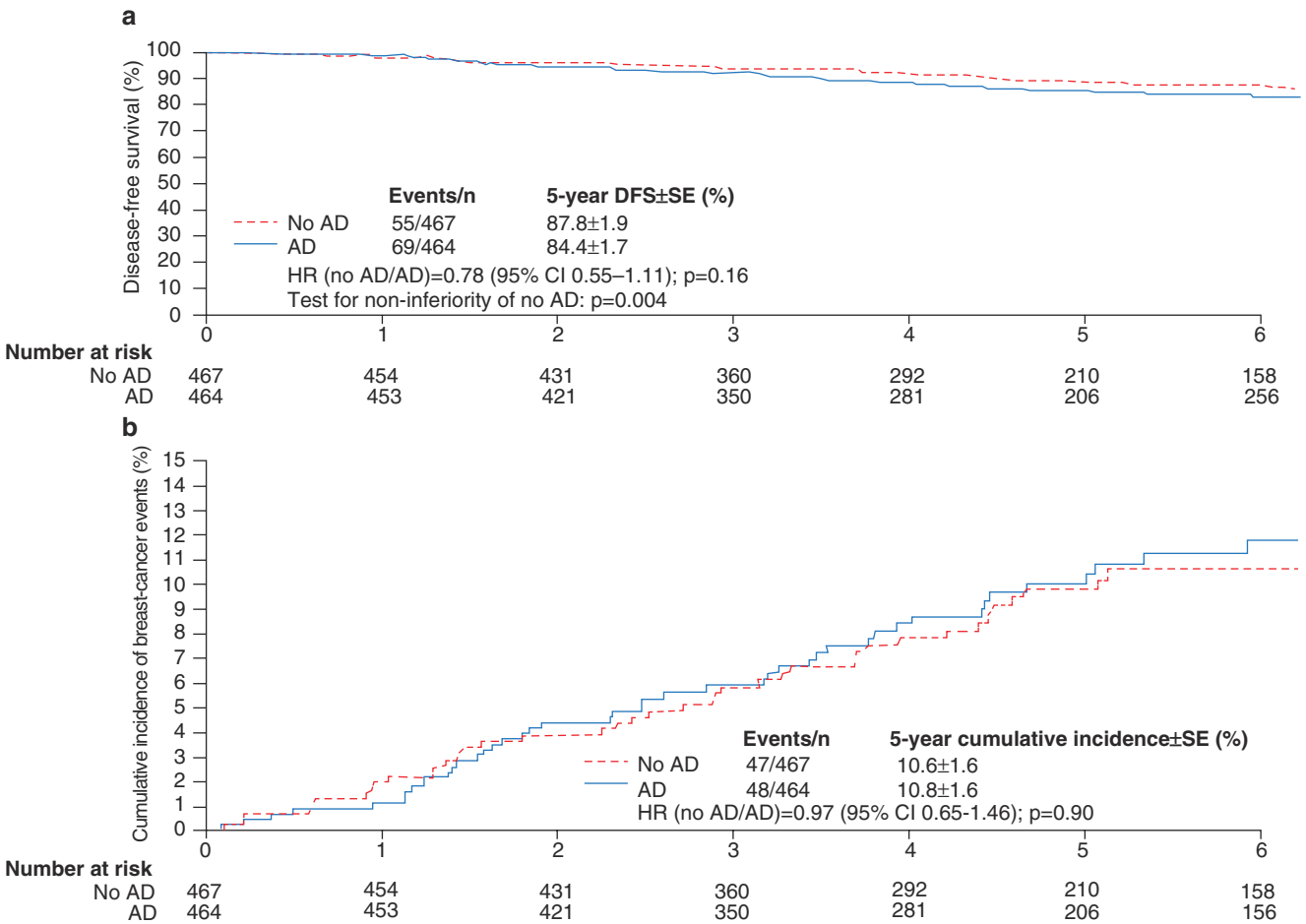

Fig. 23.8 Analysis of disease-free survival, cumulative incidence, and overall survival by intention to treat (n = 931 patients) *AD* axillary dissection. *DFS* disease-free survival. *OS* overall survival. (**a**) Disease-free survival. (**b**) Cumulative incidence of breast cancer events. (**c**) Overall survival in the intention-to-treat population of 931 patients

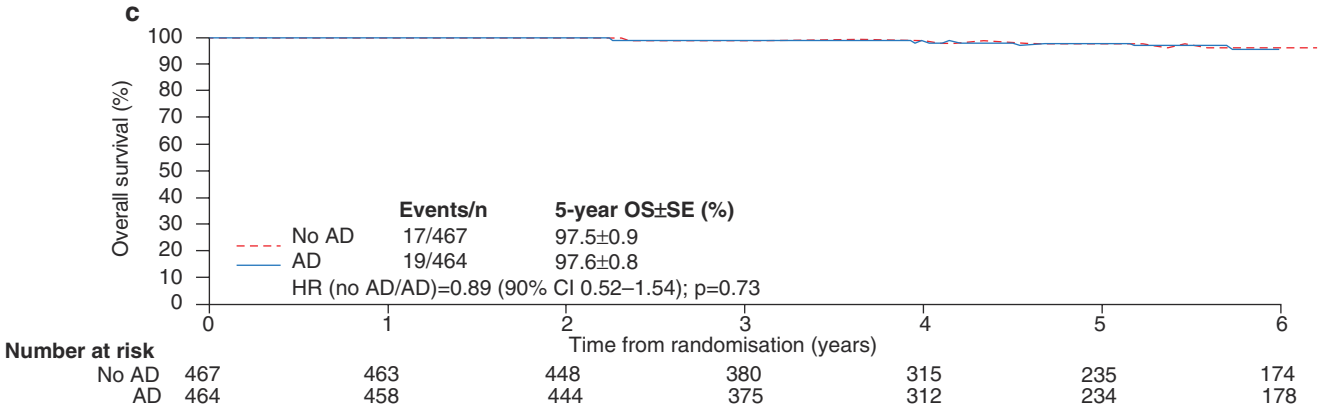

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Fig. 23.8 (continued)

studying the role of axillary treatment is the SOUND trial starting at the IEO, in Milan. Patients with pT1 tumors and negative axillary US scan are randomized to either SLN biopsy and axillary dissection if positive or to no sentinel biopsy at all. The results of this trial might completely change the approach to the axillary treatment, abandoning the sentinel node biopsy in patients with an uninvolved axilla at clinical and ultrasonographical examination.

23.7 Radioguided Occult Lesion Localization

Widespread use of mammography and ultrasound resulted in an increase in the number of nonpalpable breast lesions [32–34]. Various techniques are used to localize nonpalpable lesions and guide their removal, including wire-guided localization, carbon localization.

Radioguided occult lesion localization was developed in 1996 at the European Institute of Oncology [35]. Radioactive tracer is injected into the center of the lesion under ultrasound or mammographic control. During surgery, a gamma ray probe is used to locate the lesion and guide its removal. For malignant lesions, ROLL is used together with SNB, a technique called SNOLL [36]. In SNOLL, the patient receives two radiotracer injections: one directly into the lesion and another peritumorally. The first contains ^{99}Tc bound to colloid macroaggregates and serves to locate the lesion. In the second, the ^{99}Tc is bound to colloid microaggregates that move in the lymph ducts to reach the SN.

In recent decades, a steady improvement in imaging diagnostics has been observed together with a rising adherence to regular clinical breast examinations. As a result, the detection of small clinically occult (not palpable) lesions had progressively increased. At present in our institution, some 20% of the cases are treated when nonpalpable.

An analysis focused on 1,258 women who presented at the European Institute of Oncology [37] with a primary clinically occult carcinoma between 2000 and 2006, who underwent radioguided occult lesion localization (ROLL), axillary dissection when appropriate, whole-breast radiotherapy, or partial-breast intraoperative irradiation and received tailored adjuvant systemic treatment.

Median age was 56 years. Imaging revealed a breast nodule accompanied by microcalcifications in 9%. Microcalcifications alone were present in 17.1% of the cases, whereas distortion or thickening represented the remaining 24.6%. Most tumors were characterized by low proliferative rates (68.9%), positive estrogen receptors (92.3%), and non-overexpressed Her 2/neu (91.3%). After a median follow-up of 60 months, we observed 19 local events (1.5%), 12 regional events (1%), and 20 distant metastases (1.6%). Five-year overall survival was 98.6%.

The very high level of curability of patients whose breast carcinoma is not palpable and is discovered only with mammography, ultrasound, and MRI underlines the fundamental role of the imaging progress for the control of this disease.

23.8 Conservative Mastectomy

Conservative mastectomy might initially seem a contradiction in terms; however, if we regard conservation as the maintenance of body image, the expression is appropriate. Conservative mastectomy entails removal of breast parenchyma and saving the outer covering of the mammary gland

(subcutaneous fat, skin, and nipple), leaving the patient with a normal breast appearance. Substitution of the mammary gland for an implant is the only change made. The use of the term "conservative mastectomy" is, in our opinion, more appropriate than the alternative ones, such as "nipple and areola sparing mastectomy," which miss the notion that body image is the final objective.

Mastectomy with preservation of the skin and nipple-areola complex was first described by Rice and Strickler in 1951 for benign disease [38]. In 1962, Freeman [39] used the term subcutaneous mastectomy, and in the past 15 years, the procedure was called either nipple-sparing or total skin-sparing mastectomy. The techniques are similar to those of skin-sparing mastectomy with regard to the dissection of the skin flaps; however, additional preservation of the nipple needs a technically demanding retro-areolar dissection aiming at balancing complete removal of ducts with protection of nipple vascularization.

Location of the incision can be periareolar, with or without lateral extension, on the submammary fold, radial, or an omega pexy incision [40–42]. Periareolar incisions have the highest risk of nipple necrosis, whereas lateral radial incisions facilitate glandular dissection and access to the axilla for sentinel lymph-node biopsy. Some surgeons advocate video-assisted or endoscopic techniques with a midaxillary line incision [43, 44]. Sentinel lymph-node biopsy should always be undertaken during conservative mastectomy, with the breast incision used as access to the axilla (Fig. 23.9).

Skin flaps are created during conservative mastectomy that follow the cleavage plane within the subcutaneous fat, ensuring excision of all glandular tissue while a thin subcutaneous layer is preserved to support the vascular network [45]. The technique of flap dissection is important to provide adequate vascularization yet guarantee complete excision of ducts. During dissection from the pectoralis muscle, the fascia should be preserved. For large-breasted women, this dissection is risky and demanding, and sometimes skin reduction might be mandatory to achieve a normal-looking breast shape with acceptable ptosis [46–48]. In this case, nipple-areola complex preservation can be difficult, because final positioning of the nipple and areola after reconstruction might not be symmetrical to the contralateral.

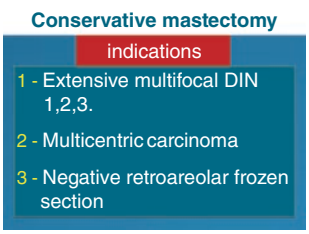

Fig. 23.9 Indications for conservative (or nipple sparing) mastectomy

The most challenging part of conservatory mastectomy is the subareolar excision because of the risk of nipple ischemia. Jensen [49] and Palmieri [50] both attempted to precondition the nipple-areola complex by dissecting it under local anesthesia from the underlying breast tissue several days before the mastectomy procedure, to stimulate blood flow from the peripheral skin. This approach has the advantage of retroareolar biopsy before mastectomy. Routinely, at the time of conservative mastectomy, the duct bundle and all retroareolar tissue are removed, and the specimen is analyzed by frozen section. This method is reliable, but some clinicians advocate the use of imprint cytology instead [51].

Intraoperative radiotherapy of the nipple-areola complex has been implemented in some centers when the frozen section of retroareolar tissue is negative, as a risk-reducing technique for local recurrence [21, 52]. However, radiotherapy is not used in all studies, yet favorable results are reported [53, 54].

The areola and a margin of 1 cm around it are included in the 90% isodose, and the dose administered must be equivalent to a fractionated dose ranging from 40 to 45 Gy [52]. In rare cases of impaired nipple vascularization diagnosed intraoperatively, external radiotherapy to the nipple-areola complex can be delivered in one session on the first postoperative day. Is radiotherapy really needed, and would a specific subgroup of patients benefit from it? A randomized trial is planned at our institute.

Heterologous implants are used extensively in breast reconstruction, and the option of fixed-volume silicone or expander implants is available. Expander implants are preferred in cases of compromised blood supply because they offer the advantage of minimal retroareolar pressure in the immediate postoperative days, when the areola is still at risk. The implant is positioned under a muscular pocket created by the pectoralis major and the serratus muscle [55]. Expanders are also preferred over fixed-volume implants, to avoid excess skin tension and flap ischemia [56, 57]. Autologous myocutaneous flap reconstruction is preferred for large-breasted women with a large skin envelope after glandular excision (Fig. 23.10).

Between March 2002 and December 2011, 2,487 patients underwent conservative mastectomy at the European Institute of Oncology. Exclusion criteria were nipple retraction, bloody discharge from the nipple, inflammatory changes of the breast, Paget's disease, and previous radiation therapy. Furthermore, tumor size needed to be less than 4 cm in diameter, and distance from the nipple-areola complex to the tumor had to be at least 2 cm. Clinical lymphadenopathy was not criterion for exclusion. A series of 934 women underwent surgery between March 2002 and December 2007 with a median follow-up of 50 months. Five-year overall survival was 96.4%, and women with invasive cancer had 5-year survival of 95.5%, and a 5-year cumulative incidence

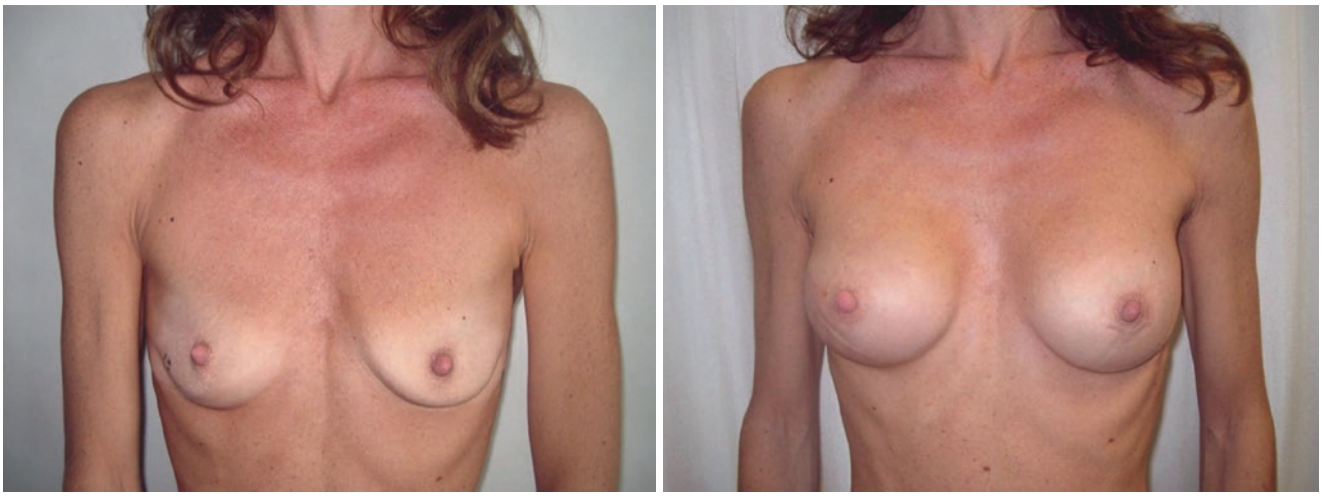

Fig. 23.10 Forty-one year-old patient before and after nipple sparing mastectomy (right breast)

of breast-related events was 14.7%. Patients with ductal intraepithelial neoplasia has 5-year overall survival of 100%. The high survival rates of our series suggest that conservative mastectomy combines safety with good cosmesis [58].

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The Conservative Mastectomy

24

Alberto Luini

24.1 Introduction

Mastectomy is the complete removal of breast gland during a surgical act: this kind of surgery seems not reliable when included in a list of conservative treatments. In fact, mastectomy is not conservative at all, but the recent evolution of breast cancer surgery has made this radical approach more conservative with the new techniques that spare a portion of the skin and mainly the nipple and areola complex (NAC).

The NAC removal in the mastectomy technique has always been needed to reduce the risk of local relapse of disease in this area, due to the fact that duct terminations are into the nipple and behind the NAC the portion of breast gland can be equally affected by all the other anatomic areas. Unfortunately, this removal has a deeply negative impact on the cosmetic outcome of mastectomy and on patients' quality of life. The absence of NAC on a reconstructed breast represents a worsening of the cosmetic result even if the reconstruction with plastic surgery has had a perfect outcome.

Patients who must undergo mastectomy (around 30% of breast cancer patients) are always worried about the mutilation they are going to receive, and this mutilation becomes particularly evident in the absence of the NAC: the breast shape can be restored and sometimes ameliorated, but the lack of the nipple and areola is difficult to repair. Of course, the plastic surgery repairs also this kind of damage with subsequent interventions, but the feeling of mutilation exists and can remain for several months.

At the European Institute of Oncology in Milan, we developed a modified mastectomy technique, and we named it *nipple-sparing mastectomy*, putting together the concept of skin sparing applied to the nipple and areola anatomic area and the occasional adoption of radiotherapy with electrons (possibly delivered during surgery with intraoperative radiotherapy with electrons ELIOT) to reduce the risk of local relapse of breast cancer. Fundamental requisite for the correct procedure is the negativity of retro-areolar breast tissue examined by histopathology during surgery.

24.2 The Nipple-Sparing Mastectomy

The nipple-sparing mastectomy is a mastectomy that removes the entire breast gland and preserves the NAC after adequate intraoperative histopathology on the retro-areolar breast tissue to exclude the presence of microscopic disease (Fig. 24.1).

This kind of surgery can be indicated to patients affected by extensive intraepithelial lesions and/or invasive breast carcinoma not located in the central quadrant of the breast and with no high risk of local relapse in the nipple area.

When we started studying nipple-sparing mastectomy, we decided to adopt two main procedures to reduce the risk of local relapse in the nipple area (after adequate selection of patients, of course):

- Intraoperative complete histopathology to the retro-areolar tissue
- ELIOT at a dose of 12 Gy to the NAC

The intraoperative histopathology on the retro-areolar tissue removed during surgery is mandatory: to preserve the NAC, we must be sure that the pathologist did not find tumor cells in the portion of the breast gland located exactly behind the nipple. Regarding ELIOT, at the beginning of our experience, we wanted to reinforce the protection to the preserved NAC by giving a dose of electrons during surgery, but subsequent analysis confirmed that a deep pathological examination of the tissue behind the NAC can guarantee extremely good results even with the avoidance of radiotherapy [1–3].

The technique of this surgery is comparable to a subcutaneous mastectomy, and great care should be taken not to compromise the blood and lymphatic flow behind the nipple: the necrosis of the nipple is, in fact, one of the main undesired

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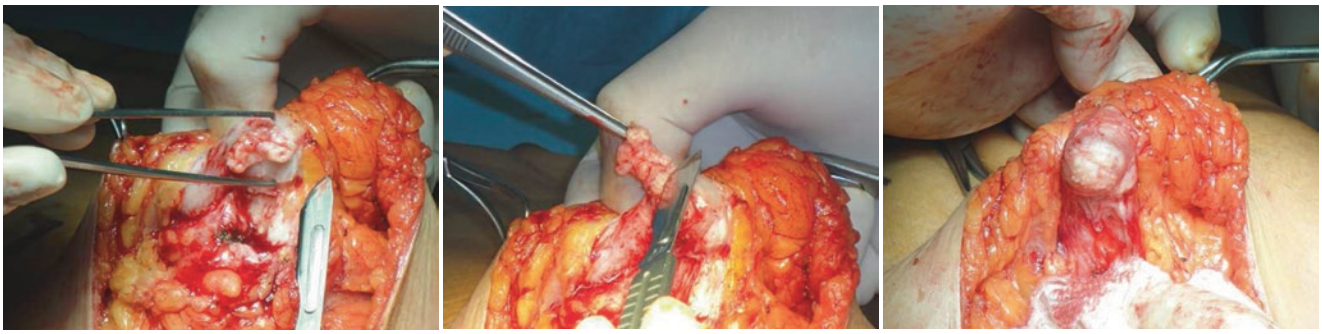

Fig. 24.1 Three phases of the removal of retro-areolar tissue to be examined for histology. In the last image, the retro-areolar tissue has been completely removed

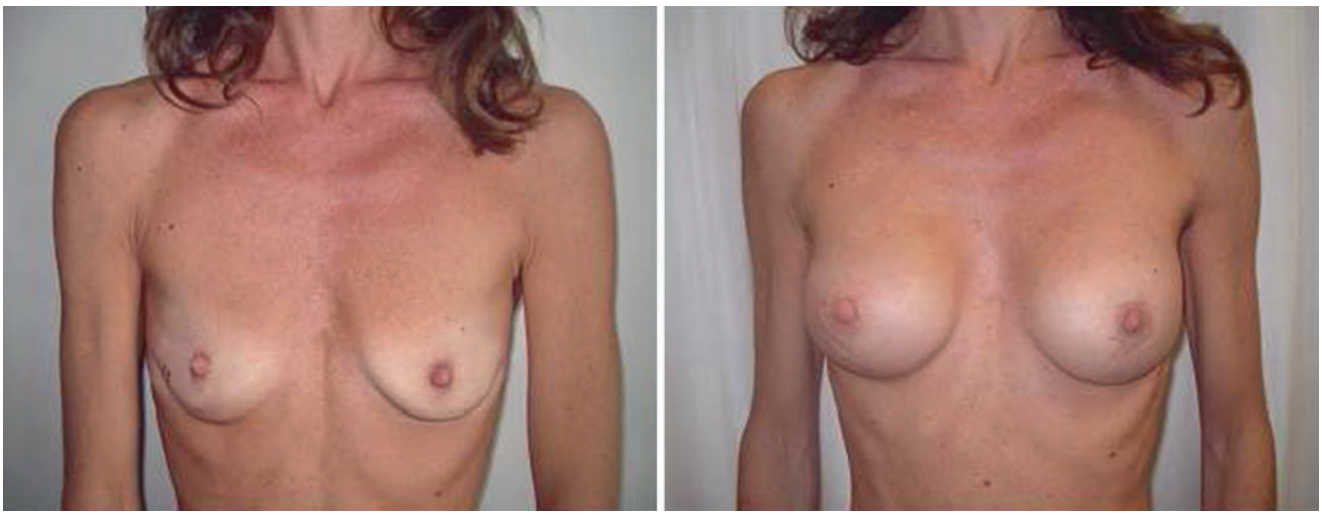

Fig. 24.2 Patient with right breast cancer before (image on the *left*) and after (image on the *right*) nipple-sparing mastectomy to the right breast and prosthesis implant in the left breast

effects of this operation together with the partial or complete loss of skin sensitivity at the nipple. Good surgical experience is crucial to obtain a representative specimen of the breast tissue behind the NAC, to be sent to the pathologist for the intraoperative examination, and the need of preserving blood nourishment to prevent an eventual necrosis [4, 5].

To decide the type of incision and the skin removal over the tumor, it is important to know the distance of the tumor from the skin or, even better, the distance among the tumor and the superficial overlying fascia (we can obtain this element with the imaging): the skin removal is suggested when this distance is less than 5 mm. Another indication to remove the skin could be a previous excisional biopsy of the tumor.

An accurate patient anamnesis is crucial to determine the presence of specific risk for skin necrosis at the NAC, such as skin pathology or defects, smoke habit, diabetes, and cardiovascular diseases.

The close cooperation between breast surgeon and plastic surgeon is mandatory (Fig. 24.2).

At the European Institute of Oncology, we are also studying a robotic approach: the nipple-sparing mastectomy is under evaluation with the aid of the Da Vinci technology. High proportion of patients with newly diagnosed early-stage breast cancer in Europe and the USA undergo mastectomy, and more conservative approach should be studied to improve quality of life of patients who need unavoidable mastectomies. Despite the lack of a natural cavity needed for endoscopic viewing, applications of robotic surgery have recently emerged for superficial organs such as in the fields of thyroidectomy, oropharyngeal surgery, and plastic and reconstructive surgery. However, it has never been applied in breast cancer except for a feasibility and safety study conducted by Toesca et al. [6] that firstly published the robotic technique considering the first three cases. In these initial cases of robotic nipple-sparing mastectomies and immediate robotic reconstruction with implant, we found two main advantages such as the robotic optical vision and the minimal invasiveness. The two main limitations noticed in this

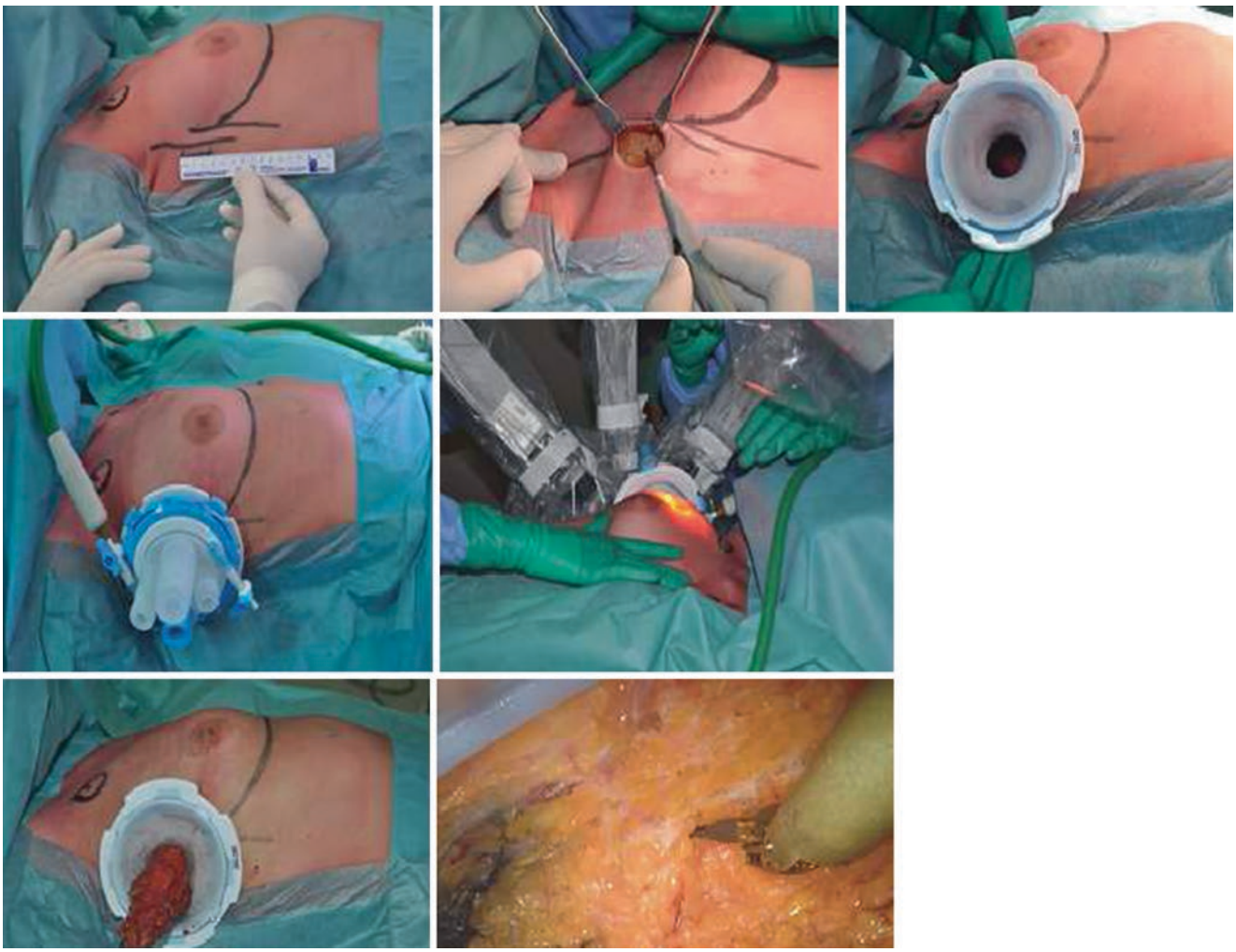

Fig. 24.3 Robotic technique

initial experience were the duration of operating time and the additional costs related to the operation. The limitations of the applicability of robotic surgery to the breast, such as operating time and costs, might be offset by the advantages we observed such as better vision and minimally invasive approach with an anatomically more respectful mastectomy. The same research has continued on breast cancer evaluating not only the feasibility and safety and oncological outcome but also patient satisfaction and quality of life of patients who undergo to this robotic approach (study ahead of print) (Fig. 24.3).

Our experience and a good amount of external trials have already confirmed that a well-conducted nipple-sparing mastectomy is effective in treating selected cases of breast carcinoma with no significant difference in the risk of local relapse of disease, NAC local relapse, and overall and disease-free survival among the new technique and the radical mastectomy that removes the NAC [7–12]. Regarding the rate of side effects, the NAC necrosis and the loss of skin sensitivity in that area are the most frequent events, but they should be compared with the total absence of NAC of the standard approach, so the impact on patients' quality of life of the nipple-sparing mastectomy seems absolutely better whenever the technique is feasible.

The reconstruction technique is chosen depending on breast volume and shape.

24.3 Indication and Present Guidelines

Retrospective trials on skin-sparing mastectomy showed a great variability on the occult involvement of the NAC by tumor cells, but the most relevant reports demonstrate a percentage of this involvement not superior to 20%: these data become lower (to 2.6%) when the lesion is at least at a 2 cm—distance from the NAC.

Patients candidate to nipple-sparing mastectomy have the following characteristics:

- Invasive or noninvasive neoplasm not involving the NAC at the evidence of the clinical and instrumental examinations
- Breast with small or medium dimension with minimal or intermediate ptosis

Absolute contraindications are:

- Clinical and instrumental evidence of pathological involvement of the NAC
- The presence of pathological nipple discharge
- Paget disease
- Inflammatory breast cancer

Relative contraindications are:

- Previous radiotherapy to the breast or prevision of radiotherapy
- Previous periareolar surgery
- Smoke habit, diabetes, cardiovascular disease.

We do not consider contraindications:

- Patient's age
- Close proximity of the tumor to the skin (not NAC), but the skin must be removed during surgery
- Previous neoadjuvant therapy
- Tumor dimension[1]
- Multifocality and multicentricity
- Tumor histology
- Lymph node status

In some selected cases, radiotherapy could have an alternative role to the NAC removal when the final histology demonstrates free margins with a distance disease NAC less than desired. The electron treatment can be postponed during 48–72 h after surgery.

[1]These elements increase the risk of NAC involvement, but they lose their significance if the distance from NAC is maintained.

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Surgical Treatment of Local Recurrence in Breast Cancer Patients

25

Jose Vila, Francisco Ripoll, and Oreste D. Gentilini

25.1 Introduction

25.1.1 Definition of Local Recurrence

Local recurrence is defined as the reappearance of an invasive tumor in the ipsilateral preserved breast after Breast-conserving surgery (BCS), or a breast cancer recurrence in the skin, subcutaneous tissue, muscle, or underlying bone after mastectomy. When a local recurrence occurs after a conservative approach, it is called ipsilateral breast tumor recurrence (IBTR) and chest wall recurrence (CWR) when it occurs after mastectomy [1]. Local recurrence tends to occur later after a conservative procedure than after mastectomy, especially in those patients treated by endocrine or chemotherapy [2–4]. Chest wall recurrences are generally diagnosed during physical examination. In contrast, IBTRs are more frequently detected during posttreatment mammographic surveillance [5].

A local failure is considered a marker of aggressiveness as it has been demonstrated to be associated with a three to five times greater risk of distant metastasis and represents the main cause of breast cancer-related death [6]. In the US National Surgical Adjuvant Breast and Bowel Project B-06 (NSABP B-06) study, Fisher et al. demonstrated that patients with IBTR have 3.41 greater risk of developing distant metastasis than patients who do not develop such recurrences [7]. Several factors have been associated with the reappearance of breast cancer such as initial surgery and use of adjuvant therapies (chemotherapy, hormonal therapy, and radiation therapy), residual tumor burden, clinical and pathologic characteristics, and biologic markers of the primary breast tumor [8, 9]. Recently, Shangani et al. updated and validated a web-based predictive model, called IBTR! Version 2.0, to estimate individualized risk of IBTR after breast-conserving therapy. This online tool incorporates seven variables that are routinely assessed and has been associated with increased risk of local failure after conservative surgery, such as age, margin status, lymphovascular invasion (LVI), size tumor, grade, and use of chemo- and/or hormone therapy [10]. This nomogram can be easily implemented in many centers worldwide and may help guide decisions about adjuvant therapies in patients according to their risk of IBTR.

A recent analysis of 86.598 patients from 53 randomized clinical trials showed that isolated local-regional recurrences are now an uncommon event. Advances in the management of early stage breast cancer have significantly reduced the rate of local-regional recurrence from approximately 30% in past studies to 5–15% in recent trials [11]. Even though a rare event, local recurrences are associated with an increased risk of distant metastasis, especially early recurrences occurred within the first 2 years after primary treatment. So, a careful restaging evaluation including a complete blood test, radionuclide bone scan, breast magnetic resonance imaging (MRI), a total body positron emission tomography (PET), and/or chest, abdomen, and pelvis computed tomography (CT) scan may be appropriate in all patients with local recurrence after BCS or mastectomy to rule out the presence of distant disease.

25.1.2 Classification of IBTR

IBTR after BCS comprises a heterogeneous group of tumors with distinct biological behavior and different rates of survival. Although a recognized standard classification for local recurrence after BCS does not exist to date, IBTR has traditionally been categorized as true local recurrence (TR) and new primary tumor (NPT). These two entities were first described by Veronesi et al. in 1995 and were classified basically by its location relative to the primary tumor. TR was defined as the regrowth of invasive disease

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at the tumor bed or the boost volume of the treated breast and NPT as a new lesion located at a different site from the primary tumor [12].

Others methods of classifying IBTRs have been proposed by different groups that have attempted to evaluate indicators of prognosis in patients with IBTR. In this sense, Huang et al. classified local recurrences after BCS as either NP or TR based on location and histology [13]. More recently, Sakai et al. have proposed a novel classification of IBTR into four different subtypes based on strict pathologic rules [14]. Initially IBTRs should be classified according to their origin as new primary or true recurrence, similar to others, but subsequently classified again according to the relationship between the IBTR and the primary lumpectomy scar, surgical margin of the primary tumor, and the presence of carcinoma in situ into the IBTR.

The importance of establishing an accurate classification of IBTR is due to the prognostic significance related to both entities [15]. A new primary tumor has been associated with a more favorable prognosis than true local recurrences. Smith et al., in a retrospective study on 130 patients with IBTR, 60 of which were classified as a true recurrence and 70 as a new primary according to the site of failure, histologic subtype, and results from the flow cytometry, assessed the prognostic value of this classification. After a median follow-up of 10.4 years, patients with NPT had significantly better 10-year overall survival, distant-free survival, and cause-specific survival than patients diagnosed with TR [16].

25.1.3 Risk Factors of Local Recurrence

As local recurrence after BCS or mastectomy has been associated with a poor prognosis, it is important to identify patients who are at higher risk of recurrence and might benefit from additional adjuvant therapies and close follow-up [17]. In this sense, several risk factors of local recurrence have been identified either in patients treated with primary conservative surgery or mastectomy [18, 19].

– *Patients treated with primary BCS*. Reasonably, the most important predictor of increased risk for IBTR in patients treated with BCS is the failure to achieve optimal local control. Optimal local control includes a margin-negative surgery (no evidence of invasive cells at the inked border on microscopic evaluation) and use of radiation therapy with or without regional nodal irradiation. Other risk factors for recurrence after BCS include (1) tumors with aggressive biology such as triple-negative tumors, high proliferation rate of Ki-67, multicentric disease, tumors with high nuclear grade, etc., (2) young age at diagnosis of primary tumor, and (3) lymphatic invasion and extensive intraductal component at the first tumor.
– *Patients treated initially with mastectomy*. The clinical risk factors associated with increased risk of local failure after mastectomy include (1) young age at diagnosis, (2) tumor greater than 5 cm, and (3) multicentric disease. Histopathologic risk factors for CWR include (1) patients with four or more positive lymph nodes, (2) positive margins, (3) high-grade triple-negative tumors, and (4) presence of lymphovascular invasion.

25.2 Surgical Treatment of Chest Wall Recurrence

The incidence of CWR after mastectomy ranges from 8 to 40% and depends on several factors such as primary tumor characteristics and the use of adjuvant therapies after mastectomy, mainly the use of postmastectomy radiation therapy (PMRT). Several studies have demonstrated that the use of PMRT resulted in better local control of primary tumors by reducing the rate of CWR by up to 70%, especially in patients with node-positive disease in whom the absolute reduction in the recurrence risk is bigger [20]. Similar CWR rates and survival outcome have been found between patients treated with conventional mastectomy versus skin-sparing mastectomy as well as comparing different types of reconstruction [21, 22]. CWR is diagnosed with concomitant distant disease in up to 30% of patients. Absolute contraindications for curative intent resection include extensive local disease with multiple skin nodules and concomitant distant metastasis. Those patients are candidates to receive systemic therapy prior to evaluate the role of salvage surgery [23]. Although CWR may manifest itself as a macroscopically extensive disease or fungating masses, often it is presented as an asymptomatic nodule in the skin or a slight erythematous rash. Hence, diagnosis requires an experienced physician with high index of suspicion, particularly in high-risk patients. Any suspicious lesion mandates a careful evaluation including biopsy and pathologic confirmation.

The surgical management of these patients is complex and requires a preoperative planning between breast surgeons and plastic surgeons to help decide on the best reconstructive option. An estimation of the extent of the disease and the need for skin grafts or rotational flaps are discussed at these meetings. In all cases, achieving clear margins is essential to provide excellent long-term local control.

For no-reconstructed breast patients with isolated CWR confined to the bed tumor or proximal to the scar, a surgical tumor resection followed by primary closure is generally feasible and oncologically safe. However, for extensive recurrence, chest wall reconstruction using coverage with

skin grafts or autologous flaps is usually needed. In some cases, chest wall resection might require resection of the ribs, sternum, and costal cartilages, and the reconstruction technique depends on the site and extent of the chest wall defect [24]. However, it has to be highlighted that the utility of such wide resection is controversial.

For patients with previous reconstructed breast, the surgical management depends on the type of reconstruction. If patients with CWR had implant-based reconstruction, removal of the implant is sometimes required, but this is not absolutely indicated and, if technically possible, implant might be left in place. In patients with flap reconstruction (transverse rectus abdominus musculocutaneous, TRAM, or latissimus flap), wide surgical resection preserving the flap may be safe in selected cases of isolated CWR [25].

Postmastectomy radiation therapy (PMRT) has been proven to be a determinant for local control of the disease in patients treated with surgical resection after CWR [26–28]. Recent guidelines recommend a complete course of irradiation to the chest wall and supraclavicular and infraclavicular regions for patients initially treated with mastectomy and no prior radiation therapy. A standard dose of 50 Gy with 1.8/2 Gy fractions followed by an additional boost of 10 Gy should be applied [29]. In selected patients with previous irradiations, a second course of radiation as part of an individual multimodal treatment concept is feasible as is associated with acceptable acute and late morbidity and encouraging local control. Wahl et al. reviewed the toxicity and clinical outcomes of a second course of radiation in a multi-institutional study on 81 patients with CWR who underwent repeat radiation therapy of the breast. After a median follow-up of 12 months (range 1–144 months), only four patients developed grade 3 or 4 toxicity and no treatment-related deaths occurred [30]. A similar report was published by Hannoun-Levi et al. [31] evaluating the effect of chest wall re-irradiation using brachytherapy. The study included 32 patients with local recurrence after BCT treated with mastectomy followed by low- or high-dose rate interstitial brachytherapy. At a median follow-up of 22 months, the second local recurrence rate was low (3%), but the distant metastasis rate was 28%. Grade 3 late skin toxicity was observed only in two patients with no grade 4 toxicity.

25.3 Surgical Treatment of Ipsilateral Breast Tumor Recurrence

Though there have been significant advances in the management of breast cancer patients, the optimal treatment for patients with local recurrence after a conservative surgery is still controversial, and there are no data from randomized trials to guide treatment decisions. According to recent studies, 15% of patients with IBTR after BCT are considered inoperable due to the extensive local disease or concomitant distant metastases. The remaining 85% of patients who are diagnosed with operable tumor recurrence are candidate to surgery. International guidelines still recommend mastectomy as the standard approach for IBTR after BCS [32, 33]. However, several retrospective studies comparing mastectomy and repeating BCS have reported similar survival outcomes between both procedures, suggesting that a second conservative approach may be recommended in selected patients (Table 25.1).

Table 25.1 Outcome by surgical procedure after ipsilateral breast local recurrence

Author (ref.)	Total patients	BCS (n)	M (n)	Follow-up (months)	2nd LR (after BCS)	BCS 5y/10y DFS	BCS 5y/10y OS	M 5y/10y DFS	M 5y/10y OS
Kurtz et al. [34]	118	52	66	84	23%	NA	79%/64%	NA	68%/54%
Dalberg et al. [35]	85	14	65	NA	12.5%	67%/–	NA	88%/–	NA
Salvadori et al. [36]	191	57	134	73 (1–192)	19%	70.2%/–	85%/–	56%/–	70%/–
Alpert et al. [37]	146	30	116	244	7%	–/69.5%	–/58%	–/61.3%	–/65.7%
Fodor et al. [38]	44	28	16	NA	28%[a]	NA	–/81%	NA	–/81%
Chen and Martinez [39]	747	179	568	6	14.8%	NA	67%/–	NA	78%/–
Lee et al. [40]	131	23	108	NA	NA	NA	93.3%/–	NA	85.8%/–
Kolben et al. [41]	170	58	112	49	22.4%	57.3%/–	84.7%/–	61.9%/–	72.6%/–
Yoshida et al. [42]	102	51	51	55	19.3%	83%/–	82%/–	94%/–	92%/–

Abbreviations: *BCS* Breast-conserving surgery, *M* mastectomy, *LR* local recurrence, *DFS* disease-free survival, *OS* overall survival, *y* year
[a]2nd LR rate following salvage excision or mastectomy

Table 25.2 Suggested selection criteria for second Breast-conserving surgery

Suggested selection criteria for a second breast-conservative approach
Age ≥50 years
Small cancer ≤2 cm
Late recurrence (>48 months)
Absence of multifocality and/or multicentricity on clinical and conventional imaging examination including breast MR
Desire of the patient for conservative approach
Acceptable cosmetic results

From Vila J, Garcia-Etienne CA, Gentilini O. Conservative surgery for ipsilateral breast tumor recurrence. J Surg Oncol 2014; 110:62–67 [43]

Patient selection criteria are crucial and represent a guide to selecting the best candidates for consideration of second conservative surgery. Vila et al. in a recent review proposed six clinical conditions that should be taken into account to help select the subset of patients who might benefit of less radical surgery with acceptable long-term survival outcome and local-regional control [43]. The selection criteria are listed in Table 25.2. Careful restaging workup of patients with local recurrence after BCS is mandatory to exclude distant disease and to identify patients who can be managed with curative intent.

25.3.1 Mastectomy

Although breast cancer treatment is becoming more conservative, mastectomy still remains the standard treatment for ipsilateral breast tumor recurrence after breast-conserving surgery [44]. Mastectomy for IBTR provides excellent local control that ranges from 69 to 98% [45]. The benefit of chest wall or regional nodal irradiation in patients treated with post-recurrence mastectomy has not been addressed but generally is not recommended in previously irradiated patients. However, regional nodal irradiation should be considered in high-risk patients who initially did not receive irradiation of the regional nodes. Ideally, mastectomy should be followed by immediate breast reconstruction using either a breast implant or autologous tissue.

25.3.2 Second Breast-Conserving Surgery ± Radiation Therapy

Retrospective studies addressing the role of a second conservative procedure have shown similar survival outcome when compared with mastectomy. Clinical outcome of patients treated with a second lumpectomy with or without re-irradiation is listed in Table 25.3. The largest series evaluating second BCS alone for in-breast local relapse was reported by Gentilini et al. This retrospective study evaluated 161 patients who underwent a second conservative alone approach after BCS and whole breast irradiation [55]. With a median follow-up of 81 months after IBTR, the 5-year overall survival was 84% (95% confidence interval [CI] 78–89) and a 5-year cumulative incidence of a further local reappearance of the tumor of 29%. This rate was lower than previous series and may be related to the small tumor size in the second BCS cohort (60% of the tumors were <1 cm). However, for patients with the diagnosis of a small relapse (<2 cm) occurring late after primary treatment (>48 months), the cumulative incidence of a further in-breast event was 15%. This finding suggests that motivated patients with the early diagnosis of a second primary tumor might be considered for a repeat BCS as an alternative to mastectomy. The local control after repeat BCS in the published series is similar to the outcome achieved by conservative surgery alone without radiotherapy. Therefore, due to improved radiotherapy treatments, a second course of radiation treatment should be carefully considered in those patients undergoing a second conservative procedure for the treatment of IBTR [56].

The largest experience evaluating the combination of a second conservative procedure followed by radiation therapy in patients with previously irradiated breast exists for multicatheter brachytherapy. As shown in Table 25.3, the addition of a second course of irradiation resulted in better local control. For patients treated with second lumpectomy alone, the

Table 25.3 Outcome of BCS ± re-irradiation for ipsilateral breast tumor recurrence

Author (ref.)	Total patients	Re-irradiation (type)	Follow-up (months)	2nd LR	5-year OS
Maulard et al. [46]	38	Yes (BT)	48	21	55
Voogd et al. [5]	16	No	53	38	NA
Deutsch [47]	39	Yes (EBRT)	51	20.5	77.9
Resch et al. [48]	17	Yes (BT)	59	24	88
Kraus-Tiefenbacher et al. [49]	17	Yes (IORT)	26	NA	94
Chadha et al. [50]	15	Yes (BT)	36	7	100[a]
Trombetta et al. [51]	26	Yes (BT)	38	4	88.5[b]
Guix et al. [52]	36	Yes (BT)	89	3	96.7[c]
Ishitobi et al. [53]	78	No	40	21	NA
Kauer-Dorner et al. [54]	39	Yes (BT)	57	NA	87
Gentilini et al. [55]	161	No	81	29	84
Hannoun-Levi et al. [56]	217	Yes (BT)	47	7	88.7

Abbreviations: *ref* reference, *LR* local recurrence, *OS* overall survival, *BT* brachytherapy, *EBRT* external beam radiation therapy, *NA* not available

[a]3-year OS

[b]3.2-year OS

[c]10-year OS

second LR rate ranged from 19% to as high as 39%, while in patients treated with a second course of irradiation, the second LR rate ranged from 3 to 21%. However, OS was less influenced by the effect of the re-irradiation as similar 5-year OS rates were observed.

Several limitations have been associated with these retrospective studies such as patient selection for second conserving surgery and the fact that no prospective studies or randomized trials have been performed comparing both procedures. So, the most important question still remains unanswered: Are all patients with operable tumor recurrence forced to undergo mastectomy instead of a second conservative procedure? The answer is no.

25.4 Surgical Axillary Management in Local Recurrence of Patients with Prior BCS

Although sentinel lymph node biopsy (SLNB) has replaced axillary lymph node dissection (ALND) in primary early stage breast tumors for women with clinically negative axilla [57], the axillary surgical management of women diagnosed with IBTR is highly controversial. Widely consulted guidelines still suggest that prior axillary surgery due to oncological reasons is a contraindication to the use of SLNB as the draining lymphatic channels are thought to be disrupted caused by the fibrosis directly related to the surgery resulting in unacceptable false-negative rates [44]. However, data from several retrospective series showed that SLNB is a technically feasible and an oncologically safe procedure to restage the axilla in patients with IBTR (Table 25.4). The success rate of second SLNB ranges from 53.7 to 92.5%. The numbers of the lymph nodes removed during the first surgery is one of the most important factors for successful identification when a second SLNB is performed [58]. In case of previous axillary lymph node dissection, no further treatment of the axilla should be necessary although several studies have reported that SLNB is feasible even in these patients. However, previous ALND is associated with the lowest detection rate of sentinel nodes.

Table 25.4 Experience with the use of second sentinel lymph node biopsy in locally recurrent breast cancer

Author (ref)	*N*	Success rate of sSLNB	Percentage of extra-axillary drainages
Port et al. [58]	54	74.1% (40/54)	5.5% (3)
Cox et al. [59]	56	80.4% (45/56)	2.2% (1)
Schrenk et al. [60]	15	80.0% (12/15)	14.3% (2)
van der Ploeg et al. [61]	36	72.2% (26/36)	47% (17)
Maaskant-Braat et al. [62]	41	53.7% (22/41)	25% (10)
Intra et al. [63]	212	92.5% (196/212)	8% (17)
Uth et al. [64]	73	65.7% (48/73)	8.2% (6)
Matsumoto et al. [65]	22	81.8% (18/22)	4.5% (1)

Abbreviations: *ref* reference, *sSLNB* second sentinel lymph node biopsy

The prognostic value of axillary restaging and the role for treatment decision making process has not been fully elucidated yet. A recent study by Ugras et al. from Memorial Sloan Kettering Cancer Center evaluated the value of axillary restaging in 83 patients with local recurrence (79 IBTR and 4 CWR) and clinically negative nodes at diagnosis. Axillary surgery was performed in 47 patients and 36 patients did not undergo axillary staging. Both groups of patients were similar according to primary tumor characteristics and adjuvant therapies received; however, time to local recurrence in the non-axillary surgery group was significantly shorter (median 3.5 vs. 6.5 years; $p < 0.05$). After a median follow-up of 4.2 years, both groups of treatment had similar rates of axillary failure, non-axillary recurrence, distant metastasis, and death. The authors concluded that preoperative SLNB, although technically feasible, may not be necessary in some cases and should be confirmed in larger cohort of patients [66].

Conclusions

The surgical management of local recurrences after BCS or mastectomy requires integration of health professionals providing multidisciplinary care that comprises breast and plastic surgeons, oncologists, and radiotherapists. Factors such as time to recurrence, site of relapse, initial nodal status, and clinical characteristics of the primary tumor have been shown to predict for differences in overall survival, disease-free survival, and local-regional control. A summary of the surgical options for breast tumor recurrence is represented in Fig. 25.1.

The management of isolated CWR depends on accurate assessment of many variables, such as age, comorbidities and desire of the patients, initial treatment, and size and location of the recurrence. For patients with previously non-irradiated mastectomy, wide resection with clear margins followed by radiation therapy should be indicated. In patients who previously received PMRT, a second course of irradiation may be considered when patients have a high risk of second recurrence. In case of IBTR, motivated patients with the early diagnosis of a second primary tumor might be considered for a repeat BCS as an alternative to mastectomy. Careful preoperative workup including breast MRI should be performed for the best patient selection. A second course of radiation treatment should be carefully considered in those patients undergoing a second conservative procedure for the treatment of IBTR. Although a second sentinel lymph node biopsy has demonstrated to be technically feasible and oncologically safe, little is known about the value of axillary restaging in these patients. Further prospective studies involving patients with local recurrences after BCS and mastectomy are needed to provide solid data that help guide physicians' treatment decisions.

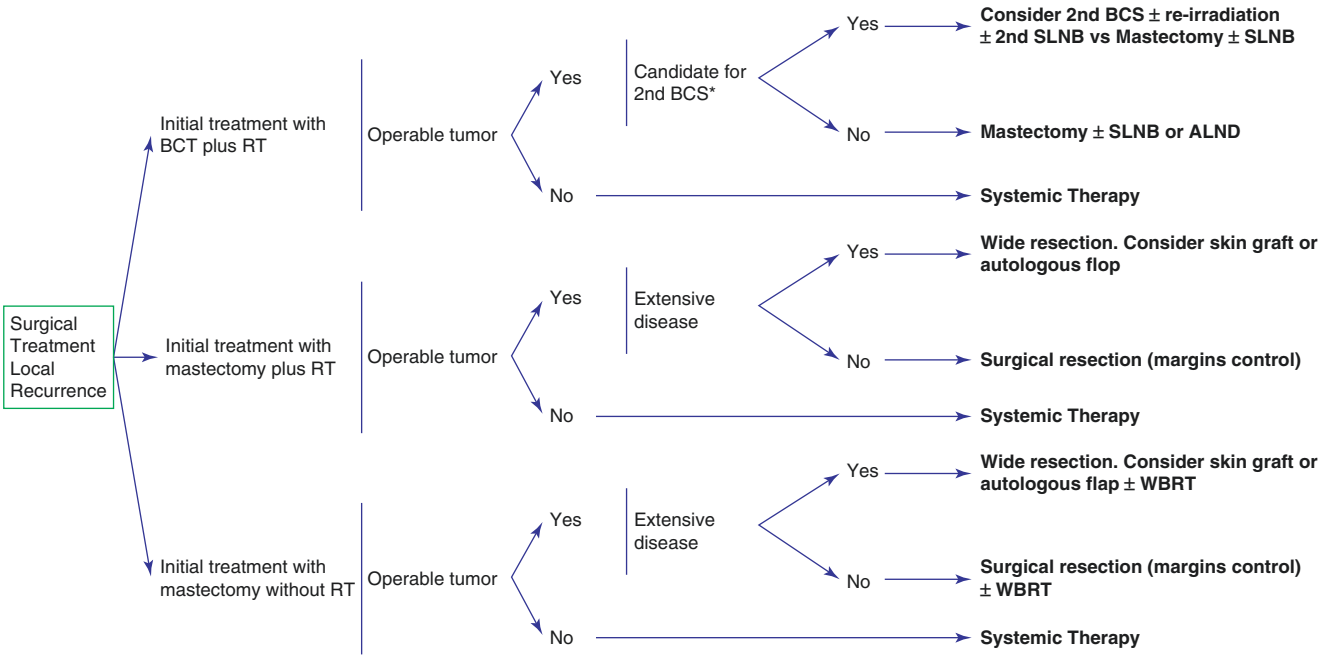

Fig. 25.1 Summary of surgical approach for breast local recurrence. Abbreviations: *BCT* breast-conserving therapy, *RT* radiation therapy, *BCS* breast-conserving surgery, *SLNB* sentinel lymph node biopsy, *ALND* axillary lymph node dissection, *WBRT* whole breast radiation therapy (including chest wall and regional nodal irradiation). *According to suggested selection criteria of Table 25.2 or following own institutions guidelines

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Management of the Axilla

26

Viviana Galimberti

26.1 Introduction

For much of the twentieth century, Halsted mastectomy was the standard treatment for operable breast cancer [1]. The operation included dissection of the axillary lymph nodes, which was reasonable since the nodes were metastatic in most patients [2]. From the early 1980s, breast-conserving surgery, flanked by irradiation of the residual breast, became an acceptable alternative to mastectomy, and by 1990, breast-conserving surgery was the preferred treatment for early breast cancer [3]. Early breast-conserving protocols included axillary lymph node dissection (AD) since most patients still presented with axillary node involvement. However there was much debate as to the utility of this procedure in patients with a clinically uninvolved axilla. As early as 1977, 5-year results of the NSABP B04 trial had shown that mastectomy patients with no clinically evident axillary disease who did not undergo AD were at no greater risk of distant disease or death than those who did receive AD [4]. Furthermore many surgeons were anxious to avoid AD if possible because of its sequelae: permanent lymphedema was relatively common [5], and pain, arm weakness, loss of arm movement, and limitation of hand movements were not infrequent [6].

On the other hand, axillary node status was recognized as a prognostic indicator in breast cancer [7], and this was important since, at that time (1980s), the only other widely used prognostic factor was the size of the primary.

In the final decade of the twentieth century, sentinel node biopsy (SNB) was introduced as a means of determining axillary status in patients with clinically node-negative disease. This proved to be a major turning point in axillary management and remains the axillary staging procedure of choice today for most women with a clinically negative axilla [8]. For a time, the introduction of SNB muted the debate on the utility of AD in patients with a clinically negative axilla, as it proved to be an accurate but minimally invasive staging procedure, yet permitted avoidance of AD in the growing proportion of patients with a pathologically negative sentinel node (SN).

However the debate on the utility of AD soon reignited as understanding of breast cancer biology increased and systemic treatments improved: in selected patients with limited axillary involvement determined by SNB, AD seems to confer no advantage, while total avoidance of axillary surgery may be justified in selected patients with an uninvolved axilla as determined by palpation, ultrasound or other presurgical investigations. In what follows current indications for axillary management will be presented in detail, followed by an outline of expected future developments.

26.2 Management of the Clinically Uninvolved Axilla

SNB is the standard approach [8] to a clinically uninvolved axilla in all patients except those with T4 disease (including inflammatory breast cancer) and those with a clinically involved axilla prior to neoadjuvant treatment. SNB was validated by a series of trials [9–14], and it is now clear that SNs can be detected in over 97% of patients, that their status predicts axillary status with about 90% accuracy, and that the axilla is site of first failure in less than 1% of cases [15].

26.2.1 SNB Technique

Several methods have been developed to identify SNs. The commonest involve peritumoral or periareolar injection of either blue dye or colloid labeled with the short-lived gamma emitter ^{99m}Tc. If blue dye is used, the surgeon searches visually for blue lymph ducts leading to blue nodes after making an incision in the axilla [16]. If the radiotracer method is used, the surgeon uses a gamma-detecting probe to guide the axillary incision and also find and remove the SNs.

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Scintigraphy may be used to identify axillary hotspots prior to surgery [17]. Some advocate use of both blue dye and radiotracer to ensure that at least one SN is always found [18]. Experience at the European Institute of Oncology is that an SN is identified in over 99% of cases using radiotracer alone [19]. Only if an axillary hotspot is not seen on scintigraphy after two radiotracer injections is the blue dye method used [20]. Recently the dye indocyanine green which fluoresces in the infrared has been used as an alternative to radiotracer [21]. It has a closely similar SN identification rate to radiotracer (≈99%) and is recommended for centers not equipped to handle unsealed radioactive materials. After indocyanine injection, fluorescence is elicited and detected by a "photodynamic eye" camera: the lymphatic drainage thus made evident is visualized in real time on a monitor. The fluorescence is followed from the injection site to the axilla, and an incision is made where the fluorescence disappears into the axilla. The fluorescent nodes are localized and excised [21].

Another recently developed SN detection technique, called SentiMag, involves injection of magnetic particles (Sienna+) and their detection with magnetic sensor. The system comprises a mains-powered base unit, a handheld probe connected to the base unit by flexible cable, and an air-operated footswitch for balancing [22]. The particles move in the lymph ducts to accumulate in the SN. The probe emits an alternating magnetic field which is absorbed by the Sienna+ particles. In turn these particles emit a magnetic field detected by the probe. Results using this method appear comparable to the radiotracer method [22].

26.2.2 Surgical Removal and Pathological Examination of SNs

If the radiotracer method is used, the gamma-detecting probe is used in the operating room to verify the presence of one or more axillary hotspots. SNB begins after the primary tumor has been removed with a 2–3-cm incision made over the hotspot (loudest audio signal on the counter connected to the probe). The isolated SNs are removed and tagged separately for frozen section examination. The axilla is then checked any for residual activity, which if present is removed. While waiting for the result of the frozen section examination, the surgeon closes the breast. If the SNs are disease-free or contain only micrometastases, the axilla is closed and not further axillary treatment given. If one or more SNs harbor metastases, complete (three-Berg-level) AD is performed (see below).

Examination of three to six SN sections is insufficient to ensure a low false-negative rate and consequently high probability that SN status reflects axillary status, so a more extensive examination of all SNs is necessary. The method used at the European Institute of Oncology is to bisect each node along its major axis and cut 15 pairs of 4-μm thick sections 50–100-μm intervals in each half node (60 sections per node). If any residual tissue is left, additional pairs of sections are cut at 100-μm intervals to completely sample the node. One section of each pair is stained with hematoxylin and eosin and examined. Only is there is any doubt as to the presence of metastasis is the second section of a pair stained by a rapid immunohistochemical method to reveal cytokeratins. SN status is then communicated to the surgeon, and depending on the result AD is performed or not performed.

26.2.3 One-Step Nucleic Acid Amplification

One-step nucleic acid amplification (OSNA) is a relatively new technique for the intraoperative analysis of SNs in breast cancer. The removed SNs are homogenized, and the number of copies of cytokeratin-19 (CK19) mRNA is determined by a rapid quantitative method shown to accurately reflect SN status as determined by pathological analysis of SN sections [23]. The number of mRNA copies correlates with extent of SN involvement (absent, micrometastatic, or macrometastatic) and permits no further axillary treatment when the copy number is low. The method has the advantage that it is reproducible and standardized and does not depend on the expertise of the pathologist, so it is particularly suited to institutes that cannot spare a pathologist for intraoperative SN examinations.

26.2.4 SNB After Breast Surgery

Previous breast-conserving surgery does not contraindicate SNB when a woman presents with disease recurrence in the breast and a clinically negative axilla. It was initially thought that surgery would disrupt the lymphatic drainage so that a new SN would be less likely to be found and would probably not receive lymph from the tumor. The European Institute of Oncology carried out 543 SNBs with radiotracer in women who had received prior breast surgery (excisional biopsy or quadrantectomy). The radiotracer was injected between the breast scar and the axilla, and the presence of an axillary initially verified with lymphoscintigraphy. An SN was identified intraoperatively in 99% of cases and was negative in 70% of them. Among the 161 patients with a positive SN, it was the only positive node in 61.5% of cases. After a median of 2 years, four cases developed axillary failure, two of which had received complete AD [24].

Even a previous mastectomy does not seem an absolute contraindication for SNB. Four patients treated at the European Institute of Oncology with total mastectomy and breast reconstruction with prosthesis developed an isolated

subcutaneous recurrence, with a clinically negative axilla. Subdermal injection of radiotracer has permitted to identify SNs [25]. Preoperative lymphoscintigraphy showed one axillary SN in three patients and two SNs in the fourth patient: SNs were positive in two patients who received AD, negative in the other two who received no further axillary treatment. Follow-up is too brief to suggest conclusions. Nevertheless it is difficult to decide where to inject radiotracer in mastectomied patients since scar tissue and fibrosis surrounding the scar are likely to impede lymphatic drainage and hence SN identification.

26.2.5 SNB After SNB and Radiotherapy

When disease reappears in the operated breast (recurrence or second cancer) after breast-conserving surgery and SNB, a new SNB is indicated if the axilla is clinically clear. Although the lymphatic system is disrupted by breast and axillary surgery, lymphatic drainage subsequently re-forms to allow identification of a new SN [26]. Even after radiotherapy the lymphatic drainage system re-forms, allowing SN identification in most patients [27]. In recurring patients, as in those treated for the first time, the prognostic information provided by SN examination is important; and the patient should be spared AD if possible. From May 2001 to December 2011, 212 patients treated at the European Institute of Oncology by breast-conserving surgery plus SNB with a pathologically clear SN experienced local reoperable recurrence. Preoperative lymphoscintigraphy demonstrated at least one new SN in 207 patients (97.7%), whereas no drainage was observed in five patients (2.3%). One or more SNs were surgically removed from 196 of the 207 patients. SNs were not isolated from the remaining 11 patients. The success SNB rate was 92.5%. Extra-axillary drainage pathways were visualized in 17 (8%) patients. The annual axillary recurrence rate after a median follow-up period of 48 months was 0.8%, and the cumulative incidence of axillary recurrence at 5 years was 3.9%. These data indicate that second SNB should be considered for patients who underwent conservative surgery and had a negative axilla and subsequently recurred locally [28].

26.2.6 SNB After Neoadjuvant Treatment

The 2014 ASCO guidelines indicate that most women with breast cancer should have SNB and reported "intermediate level evidence" that the benefits of SNB after neoadjuvant treatment outweighed the harms. However the guidelines did not recommend SNB in women with an involved axilla prior to neoadjuvant treatment, even if they became cN0 afterward. The reason given was that the false-negative rate (FNR) may range from 10 to 30%, and this was considered unacceptably high [8]. Several studies in fact found FNRs above 10% [29–32]; others however found FNRs below 10% [33–36]. Furthermore the clinical significance of a high FNR is unclear, since the early randomized trials on SNB found that while the FNR was of the order of 10% (control arms), the axillary failure rate in the SNB-only arms was of the order of 1% [10, 14].

To address this issue, a retrospective study from the European Institute of Oncology investigated outcomes in a consecutive series of patients treated between 2000 and 2010. One group of 147 patient was cN1/2 before neoadjuvant treatment and became cN0 afterward and received SNB with AD if the SN was positive. These were compared with to those in a consecutive series of 247 patients, treated over the same period, who were cN0 before neoadjuvant treatment and remained so afterward. After a median follow-up of 61 months, just one patient in each group developed axillary failure as first event; one other patient in each group developed simultaneous local plus regional failure suggesting that SNB is acceptable in cN1/2 patients who become cN0 after neoadjuvant therapy. Furthermore survival outcomes were closely similar in the two groups [37]. The 2015 St. Gallen Conference Panel [38] considered that SNB was appropriate in patients with a clinically positive axilla at presentation who downstaged after neoadjuvant chemotherapy, but that AD was required if even one SN was positive. Nevertheless FNRs remain high unless three or more SNs are examined.

26.2.7 SNB in Multicentric Disease

In multicentric breast cancer, the different disease foci may drain to different axillary nodes so a negative SN has a greater probability of being a false negative (and not reflecting the true state of the axilla). At the European Institute of Oncology, two techniques are adopted to minimize this possibility: (a) if there are two cancer foci, two subdermal injections of radiotracer are given; (b) if there are several tumor foci, a single sub-areolar injection is given, since there is evidence that the axillary nodes receive lymph from a periareolar network of superficially located lymph ducts [39]. Between June and December 2007, 337 patients with multicentric breast cancer and a clinically negative axilla underwent SNB at the European Institute of Oncology. In 100% of cases, at least one SN (median 1.7, range 1–7) was identified. A total of 138 patients with either negative SNs ($n = 134$) or isolated tumor cells in the SN ($n = 4$) did not undergo AD. There were 27 (19.5%) events in the latter group, but only three (2.2%) developed axillary disease after a median follow-up of 5 years (range 17–134 months) providing evidence that SNB is acceptable in patients with multicentric disease [40].

26.2.8 SNB in Pregnancy

Results of a dosimetry study carried out at the European Institute of Oncology indicate that the use of radiotracer to identify the SN in pregnant women is unlikely to have any adverse effects on the fetus. The study recruited 26 premenopausal, nonobese, nonpregnant breast cancer patients of median age 36.7 years scheduled for SNB with radiotracer. Scintigraphy revealed radiation (gamma rays) coming only from the injection site and the axilla. Skin dosimeters (thermoluminescent detectors) were placed on the epigastric, umbilical, and hypogastric regions to measure radioactivity that might be received by the fetus. In 23 of these patients, the absorbed dose was below that detectable by the detectors; in the remaining three patients, minimal doses (about 1000 times lower than the threshold for deterministic effects) were recorded. These findings suggest that use of the standard amount of radioactivity (12 MBq di ^{99m}Tc) to perform SNB is safe at all stages of pregnancy [41].

26.2.9 SNB in Male Breast Cancer

Only about 1% all breast cancers occur in men. Breast cancer is generally diagnosed later in men than women, and around 60% of male cases have axillary involvement at diagnosis, when SNB is not indicated. However, tumor biology, prognostic factors, and prognoses seem closely similar in the two sexes [42] (even though trials to define optimal treatments in men have not been conducted), and men with a clinically clear axilla should therefore be candidates for SNB. SNB is particularly indicated because the sequelae of AD may be more incapacitating in men as their job or lifestyle may be physically demanding [43]. Between April 1999 and January 2005, 75 men were treated at the European Institute of Oncology for breast cancer. Thirty-two with a clinically negative axilla underwent SNB, and at least one SN was found in all cases (mean 1.5, range 1–3). In six cases the SN was metastatic: micrometastatic in two, only one involved axillary node in the other four. After a median follow-up of 30 months (range 1–63), there were no recurrences or axillary failures [23].

26.2.10 SNB in Ductal Intraepithelial Neoplasia

Since ductal intraepithelial neoplasia (DIN) does not metastasize by definition [44], SNB does not seem appropriate. This is supported by a study from the European Institute of Oncology [45] which found that, in 854 cases of pure DIN (no microinvasive foci identified on definitive pathological examination), SN involvement occurred in only 1.9% of cases, decreasing to 1.4% if SNs with only isolated tumor cells were excluded. This finding and others [46] justify not performing SNB in patients with "pure" DIN.

Nevertheless there is always the possibility that DIN may harbor foci of invasive or microinvasive disease not found by preoperative biopsy. The review by Shapiro-Wright et al. [47] found that the risk of a metastatic SN was relatively high in the presence of high-risk DIN (characterized by high grade, comedo necrosis, or large size) and also when the lesion was palpable. In such cases DIN was frequently (10–38%) upstaged to microinvasive or invasive breast cancer on pathological examination. This study also found that in DIN patients scheduled for mastectomy, the SN was involved in a high proportion of cases. In fact contraindication for breast conservation is a risk factor for the presence of invasive cancer or progression to invasive cancer. Tunon-de-Lara et al. [48] also found that invasive disease was frequently underestimated by vacuum-assisted biopsy of DIN patients scheduled for mastectomy.

These findings indicate that SNB should be performed in DIN patients only when biopsy indicates invasive disease; when mastectomy is indicated; when the lesion is palpable; and when the lesion is large. These recommendations are essentially the same as those of ASCO 2014 [8].

The question remains, however: should axillary dissection be performed in DIN patients with a positive SN?

Since the Z0011 trial showed that AD is not necessary in patients with invasive breast cancer and fewer than two positive SNs treated by conservative surgery and systemic therapy [49], it would seem that AD is overtreatment in DIN patients with or without micro-invasion, provided they are scheduled for conservative breast surgery. In fact, in only 0.39–13.7% of such cases are other axillary nodes involved [49, 50]. It is therefore recommended that immediate AD not be performed if the SN is positive during intraoperative examination, in DIN patients undergoing conservative breast surgery.

26.3 Management of the Metastatic Axilla

AD remains the standard treatment if the axilla is metastatic, irrespective of whether breast-conserving surgery or mastectomy is scheduled. If an SN is positive, the AD procedure initially involves enlargement of the excision to access the SNs. If the patient has clinically palpable axillary lymph nodes, increasingly, they are confirmed as positive prior to surgery by needle biopsy, ultrasound, or both, and the surgeon proceeds to AD after removing the primary tumor. The excision is made in continuity with the breast incision when the cancer is in the upper-outer quadrant and is separate when the tumor is located elsewhere. A small lateral cutaneous flap is then prepared to allow access to the lateral margin of the

latissimus dorsi, its insertion in the humerus, the coracobrachialis muscle, and the lateral portion of the vasculonervous tract. The margin of the latissimus dorsi is isolated along its length so as to identify and prepare the blood vessels and the thoracodorsal nerve. The adipose tissue between the internal fascia of the latissimus dorsi and the surface of the chest wall is detached. At this point the long thoracic nerve (Bell's nerve) adherent to the chest wall may be observed under the muscle fascia and whose pathway runs from the high portion of the latissimus dorsi vasculonervous tract to the lower part of the serratus muscle. The medial cutaneous flap is now prepared to access the lateral margin of the pectoralis major and to identify the surface between this and the underlying pectoralis minor. By introducing a retractor, the pectoralis minor may be accessed. The adipose tissue between the two muscles, which includes the Rotter lymph nodes, is thus carefully explored, and if nodes are palpable they are removed, sparing always the thoracic acromial peduncle and the interpectoralis vessels. The coracoclavicular pectoralis ligament is now located and is displaced medial to the vasculonervous thoracic acromial fascia. The margins of the pectoralis minor muscle are identified, and, medially and laterally to the coracoclavicular pectoralis fascia, the index finger is introduced under the venter musculi. Dissection of the adipose tissue continues by uncovering the plexus brachialis and the axillary vein—the anterior surface of which is isolated. By following the vein medially, the tendon of the subclavius muscle becomes visible, thus reaching the apex of the axilla (third level) where the highest axillary lymph nodes and lymphatic vessels are located. These should be isolated carefully, excising the adipose tissue from the tendon of the subclavius muscle and pulling it downward. The lateral limit of each lymph node level should be marked by metal disks or different colored threads to facilitate pathological examination. Performed in this way, so as to spare all vascular and nervous tracts of the muscles, including the intercostal nerves, AD causes side effects in less than 6% of cases yet provides maximum possible prognostic information [51]. By contrast random biopsy of one axillary node, sampling, or removal of the first level only does not ensure disease removal, does not completely obviate the risk of lymphedema, and ignores the possibility of skip metastases, which are may occur in up to 12% of cases.

26.4 Future Developments

In a sense, the future of axillary management is already here. Trials have shown that AD does not confer any benefit in selected patients with a metastatic SN. Perhaps the most important of these was the Z0011 trial [49] which recruited 891 patients with T1-T2 disease, non-palpable axillary nodes, and macrometastases in no more than two SNs, who underwent breast-conserving surgery and whole breast irradiation. They were randomized to either AD (at least ten nodes removed) or no further treatment to the axilla. Most patients also received systemic adjuvant therapy. Overall 5-year survival was 91.8% (95% CI, 89.1–94.5%) in the AD group and 92.5% (95% CI, 90.0–95.1%) in the SNB only group. Axillary failure rates were low in both groups.

The IBCSG 23-01 trial [52] assessed whether omitting AD in patients in whom one or more SNs contained only micrometastases (foci ≤2 mm) had any effect on outcomes. Patients with a non-palpable axilla and tumor up to 5 cm in diameter were eligible; they could also be scheduled for mastectomy. They were randomized to AD versus no further axillary treatment, and after 5 years, there were no significant differences in outcomes between the groups, with axillary failure rates low in both groups.

The AMAROS trial investigated whether axillary irradiation could serve as an alternative to AD in patients with clinically node-negative disease found to have a positive SN. After a median follow-up of 6.1 years, survival outcomes did not differ between the two arms, and regional control rates were high (99.5% and 99.0%) although there were fewer side effects in axillary irradiation arm (lymphedema in 28% of AD arm vs. 14% of axillary irradiation arm patients; $p < 0.0001$) [53, 54].

Further evidence was provided by long-term results of the INT09/98 trial [55], which started the pre-SNB era. The trial randomized patients, age 30–65 years, with T1N0 disease to quadrantectomy with or without AD. A total of 517 patients were evaluated. After a median follow-up of nearly 11 years (127.5 months; interquartile range 113–141 months), neither overall nor disease-free survival differed between the AD and no AD arms. Although overt axillary disease occurred in 22/245 (9.0%) of no AD arm patients (a median of 30 months after surgery), this had no effect on survival outcomes. The authors noted that the biological characteristics of primary were an adequate guide to adjuvant treatment.

Based on the findings of these studies, the 2014 ASCO guidelines [8] and 2015 St. Gallen Conference guidelines [38] recommended that most women scheduled for breast-conserving surgery and whole-breast radiotherapy, and found to have just one to two metastatic SNs, should not undergo AD, although it might be preferable if they were given some form of systemic therapy.

The question obviously arises: if AD can be omitted in many patients with a positive SN, why should we bother to perform SNB? The ongoing SOUND trial was designed to answer this question. Patients with a clinically negative axilla but positive SN are randomized either to AD or to no further surgical treatment of the axilla. To be eligible, patients must be candidates for breast-conserving surgery and have a lesion of ≤2 cm; furthermore axillary negativity must be ascertained by palpation plus axillary ultrasound, with ultrasound-guided fine needle aspiration if a single doubtful lymph node is identified on ultrasound [56].

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Management of Intraepithelial Disease

27

Antonio Toesca

27.1 Introduction

Ductal intraepithelial neoplasia (DIN) and lobular intraepithelial neoplasia (LIN) are the new acronyms that many authors now use to replace the traditional definition of ductal or carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) of the breast, respectively. This is because some authors [1] found it difficult to accept the intraductal proliferation of tumor cells being defined as a malignant tumor and others [2, 3] consider that in these cases, the "N" and "M" categories should not be applied and that there is therefore no reason to keep them within the TNM classification, as "intraductal carcinoma" [4].

In this chapter we shall examine DIN1c to DIN3 (DCIS any grade) considering the rest of the intraepithelial neoplasias, such as flat epithelial atypia (DIN1a) and atypical ductal hyperplasia (DIN1b), as risk factors of variable magnitude in the subsequent development of "in situ" breast cancer. Considering that LIN does not require treatment by way of cancer therapy, careful observation is needed to watch for any signs of invasive breast cancer. In addition, strategies such as medication or surgery can be taken into consideration to reduce the risk of breast cancer in the future. Not so long ago, most patients with DIN presented with clinical symptoms, such as breast mass, bloody nipple discharge, or Paget's disease [5].

Today, most lesions are non-palpable and generally detected by imaging alone, due to the widespread use of mammography screening programs [6].

Issues in the management in DIN include: type of surgery performed in terms of indications for breast conservative surgery (with or without radiation therapy), indications for mastectomy with reconstruction, assessment of margins, and staging of the axilla.

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27.2 Breast Conservative Surgery With or Without Radiation Therapy

Until approximately 20 years ago, the treatment for most patients with DIN was mastectomy. Today, almost 70% of newly diagnosed patients with DIN are treated with breast preservation [7].

Multiple trials have demonstrated the feasibility and oncologic safety of breast conservative surgery (BCS) in DIN [8], and BCS is used in DIN for similar indications to those for invasive carcinoma [9, 10].

Breast conservative surgery is performed particularly for those patients with small solid masses, mammographically detected lesions, or limited microcalcification areas resulting in an extremely high survival rate and low absolute risk of local recurrence. At present, mastectomy is performed in about 30% of DIN patients, BCS without RT in about 30%, and BCS followed by RT in about 40% [11].

By the early 1990s, with the launch of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-17 trial, which specifically looked at excision versus excision plus radiotherapy (RT), BCS joined mastectomy as a standard option of care in breast cancer surgery management [12].

Clearly, the benefit of RT for decreasing ipsilateral breast tumor recurrence is well established; however, none of the most important prospective studies such as NSABP B-17, the European Organization for the Research and Treatment of Cancer (EORTC) 10853 trial, the Swedish Breast Cancer Group, and the UK Coordinating Committee on Cancer Research (UKCCCR) showed an overall survival benefit, and there was no decrease in metastasis [2, 3, 13–16].

In the current management of DIN patients, physicians are faced with the issue of whether to recommend radiotherapy and/or tamoxifen treatment to their patients in addition to surgery. To aid in this decision, a number of factors are taken into account, including patient age and tumor margins, grade, and size, but the evidence to support these and other potential features as prognostic is variable [17].

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Considering that the goal in the management of DIN is to maximize local control with the least-aggressive treatment, avoiding overtreatments and multiple important risk factors can be involved in the choice of performing radiation therapy for some low-grade low-risk neoplasia such as age, multifocality, margin status, Ki-67, and menopausal status as prognostic factors for local events [18].

The most significant predicting factor for local recurrence is the presence of multifocality. In fact, a recent meta-analysis of three randomized control trials (RCTs) and two observational studies including 3895 patients showed an increased risk of recurrence in patients diagnosed with multifocal DIN of any grade (range from 1.55 to 2.97 in RCTs and from 1.8 to 6.0 in observational studies) [19]. Moreover, a large observational study of 260 patients treated by BCS alone or BCS plus external radiation therapy concluded that multifocality is an independent risk factor for the development of ipsilateral breast tumor recurrence (IBTR) after BCS for any DIN with or without RT [20].

Although many studies have the bias of retrospective material, and often have no comparison to a radiotherapy group, several previous reports have studied the association between age and risk of IBTR after BCS for DIN.

In a study by Lagios [21], low-grade disease, not greater than 25 mm, discovered mammographically and excised with at least 1 mm margins, revealed a 12% IBTR rate at 5 years and 16% at 10 years, with no breast cancer-related deaths and no systemic recurrences for patients treated without RT [22].

Adverse prognostic factors for DIN outcomes include younger age at diagnosis, symptomatic presentation (i.e., palpable), larger tumor size, higher nuclear grade, presence of comedo necrosis, and positive margins on excision. The benefit of radiation based on the presence of these factors has been a topic of controversy. The retrospective work of Silverstein in the development of the Van Nuys Prognostic Index was the first attempt at stratifying risk and prescribing a different surgical and RT regimen for DIN according to age, tumor size, pathological classification, and margin width [23–25].

The Van Nuys Prognostic Index combines several clinicopathological factors to aid decision-making regarding the use of radiotherapy and completion mastectomy after lumpectomy. The original VNPI was introduced in 1995, and it classified DCIS cases according to nuclear grade and the presence of comedo necrosis. Subsequent revisions of the VNPI included tumor size, margin width, and patient age in the scoring system. In its current format, scores of 1–3 each are assigned for patient age, tumor size, margin width, and tumor class (the nuclear grade and presence/absence of comedo necrosis), giving a sum of 4–12, with a score of 4 being associated with the lowest recurrence risk [26]. High mammographic density (at least 75% density) has been associated with a relative risk of 2.8 (95% CI 1.3–6.1) for the development of local recurrence in patients treated with local surgical excision and radiotherapy [27].

A meta-analysis conducted by Wang et al. again reported multifocal DIN to be associated with an increased risk of ipsilateral recurrence compared with unifocal tumors (overall risk estimate 1.95, 95% CI 1.59–2.4063) [28].

In conclusion, the use of RT for patients treated with BCS is still a matter of debate. In a recent Saint Gallen Consensus Conference, the majority of the panel supported radiation after complete excision of ductal carcinoma in situ (DIN) but was prepared to consider its omission for some elderly patients and for those with low-grade low-risk DIN [29].

27.3 Mastectomy and Reconstruction

In the past, when mastectomy was common, reconstruction was uncommon; if it was performed, it was generally done so as a delayed procedure. Today, reconstruction for patients with DIN treated by mastectomy is common; when it is performed, it is generally done immediately, at the time of mastectomy.

The evolution of mastectomy techniques enables patients requiring mastectomy and patients undergoing risk-reducing surgery to benefit from advances in oncoplastic surgery, with improved cosmetic outcomes and reduced psychological impact.

In the past, when a mastectomy was performed, large amounts of skin were discarded. Today, it is considered perfectly safe to perform a skin-sparing mastectomy for DIN and in many instances, nipple-areola sparing mastectomy.

In the past, there was little confusion. All breast cancers were considered essentially the same, and mastectomy was the only treatment. Today, all breast cancers are different, and there is a range of acceptable treatments for every lesion. For those who choose breast conservation, there continues to be a debate as to whether radiation therapy is necessary in every case. These changes were brought about by a number of factors. Most important were increased mammographic utilization and the acceptance of breast-conservation therapy for invasive breast cancer.

The widespread use of mammography changed the way DCIS was detected. It also changed the very nature of the disease detected, by allowing us to enter the neoplastic continuum at an earlier time. Until the 1980s, the treatment for most patients with any form of breast cancer was generally mastectomy. Since that time, numerous prospective randomized trials have shown an equivalent rate of survival with breast conservation therapy for selected patients with invasive breast cancer. On this basis, it made little sense to continue treating a lesser disease (DIN) with mastectomy while treating the more aggressive invasive breast cancer with breast preservation.

Recently, numerous investigators have assessed the value of preoperative MRI in DIN, and today the debate centers on whether or not the addition of MRI to conventional mammograms and ultrasound in the preoperative evaluation of DIN would result in a better prediction of disease extent and thus potentially improve the rate of mastectomies. Doyle et al. [30] clearly stated that they saw a statistically significant improvement with concordance between the predicted and pathologically proven extent of DIN when using a mammography-MRI combination, compared with using mammography alone. They conclude that the addition of MRI to mammography in preoperative evaluation for DIN does improve concordance with the final pathology. This is a promising result in terms of the potential for MRI to aid decision-making in DIN-diagnosed patients, given that one of the key preoperative decisions is whether or not one should proceed with BCS or with mastectomy.

27.4 Assessment of Margins

The definition of positive margins has varied widely, making data synthesis challenging. In one meta-analysis of 4660 patients in 21 studies who underwent partial mastectomy and radiation for DIN, recurrence rates were as high as 10% and showed a correlative increase in rates of recurrence with reduced thresholds for margin positivity [31].

Yet, Houssami et al. found that when the data were adjusted for the administration of adjuvant therapy, there was no significant decrease in local recurrence rates for 1-, 2-, and 5-mm margins [32, 33].

The overview of the four prospective randomized trials of RT for DCIS reported that negative margins are associated with a lower risk of recurrence [15].

Solin et al. [34] reported that in a multivariable analysis of 1003 women with mammographically detected DCIS treated with BCS and RT, and median follow-up of 8.5 years, margin status and age were the only statistically significant factors associated with recurrence. Compared with negative margins, positive margins (tumor on ink) had an HR of 3.35 (P¼ 0.00035), and close margins (defined as <2, <2–3, or <3 mm) had an HR of 1.9 (P¼ 0.03). As well demonstrated by Shaikh et al. [35] in a large series of women with DIN, where numerous factors were controlled for, they found that margin width was strongly associated with risk of recurrence for women undergoing BCS who do not receive RT. In contrast, they found no association among those who do receive RT, demonstrating a differential association of margin width and recurrence, depending on adjuvant treatment. These results support the conclusion that obtaining wider negative margins may be important in reducing the risk of recurrence in women who choose not to undergo RT or some adjuvant systemic therapy and may not be necessary in those who receive RT.

27.5 Staging of the Axilla

Over the past decade, sentinel lymph node biopsy has widely replaced axillary lymph node dissection as the preferred method of nodal staging for breast cancer. In recent years new data confirm that sentinel node biopsy for DIN should be limited primarily to those patients who require mastectomy. While earlier studies suggest a number of possible indications such as high-grade tumor, large lesion, palpability, inability to rule out invasion, etc., the percentage of micro-invasion in the resected specimen and therefore consequent percentage of positivity of the sentinel lymph node are described as less than 1% [36].

With BCS, the risk of second surgery after accidental diagnosis of infiltrating carcinoma is very low. In the event of invasion after BCS, a sentinel node biopsy is always technically feasible. The situation is different when mastectomy is performed considering that a second surgery on the axilla could be technically more problematic.

In 2014, ASCO updated recommendations on the use of sentinel node biopsy for patients with DIN [37] stating that sentinel node biopsy is recommended when mastectomy is performed. Concerning the staging of the axilla for breast conserving surgery, the panel also recommended: (1) sentinel node biopsy only for minimally invasive breast cancer demonstrated on the core needle biopsy, (2) for a lesion highly suspicious of invasive cancer, or (3) in case of an area of DCIS on imaging >5 cm.

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Breast-Conserving Surgery After Neoadjuvant Therapy

28

Mahdi Rezai and Stefan Kraemer

28.1 Introduction

Early randomized trials of the addition of neoadjuvant chemotherapy (NACT) to the treatment regimen of patients with breast cancer failed to demonstrate an improvement in overall survival compared with conventional adjuvant therapy; nevertheless, the increased opportunities for breast conservation, owing to downstaging of the primary tumour, and enthusiasm regarding the potential to tailor systemic therapy based on responses observed in the neoadjuvant setting, resulted in the adoption of this approach as a useful clinical tool. That the effectiveness of NACT varies by molecular subtype is becoming increasingly clear, and although the potential of tailoring adjuvant systemic therapy based on treatment response before surgery remains to be realized, the increasing rates of pathological complete response following NACT have had a considerable impact on locoregional treatment considerations. For example, NACT reduces the need for mastectomy and axillary lymph node dissection, thus decreasing the morbidity of surgery, without compromising outcomes. However, selection of the ideal candidates for preoperative chemotherapy remains critical, and personalizing local therapy based on the degree of response is the subject of ongoing clinical trials. The concept of *targeted breast surgery* is a systematic model of surgical techniques for breast conservation after NACT with optimized local outcome and aesthetic results for the patients.

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28.1.1 Neoadjuvant Chemotherapy (NACT)

Preoperative or neoadjuvant chemotherapy (NACT) was initially used in the treatment of patients with locally advanced breast cancer (T4a–T4d disease), after historical series of patients with inflammatory breast carcinoma (T4d disease) and other T4 breast tumours who were treated with initial surgery demonstrated high rates of local recurrence and poor survival [1, 2]. The demonstration in the 1970s that adjuvant chemotherapy improved both disease-free survival and overall survival of women with lymph node-positive breast cancer [3, 4] led to a number of studies examining the role of NACT in locally advanced breast cancer. The results of early studies of NACT indicated a prolongation of disease-free survival and overall survival compared with historical controls [5, 6], coupled with the observation that major reductions in tumour volume occurred in 60–80% of patients treated [7], providing the rationale for clinical trials of this approach in earlier-stage operable breast cancer. The primary aim of these studies was to determine if NACT, through prompt treatment of micrometastases, improved survival compared to chemotherapy given postoperatively. However, a meta-analysis of nine randomized studies, comprising a total of 3946 patients, found no significant survival difference between patients who received NACT and those who received adjuvant therapy, with a summary risk ratio of 1.0 (95% CI 0.90–1.12) [8]. Although this lack of survival difference has persisted in more recent studies [9], a number of benefits of NACT have nevertheless emerged, including increased opportunity to perform breast-conserving surgery (BCS) and a reduced need for axillary lymph node dissection (ALND) [10]. Additionally, the achievement of pathological complete response (pCR) to NACT has emerged as a powerful prognostic factor [11]. The acceptance by the FDA of pCR rate as a criterion supporting the approval of new drugs [12], together with the other benefits discussed, suggests that the use of NACT will continue to increase. This paradigm shift raises a number of important questions regarding appropriate approaches to local therapy for breast cancer, as the

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guiding principles for surgery and postoperative radiotherapy in use today were developed based on the findings of trials in which surgery was the initial treatment modality.

28.1.2 NACT and Breast-Conserving Surgery (BCS)

A meta-analysis of 14 prospective randomized trials of neoadjuvant versus adjuvant chemotherapy in a total of 5500 patients with breast cancer demonstrated that NACT was associated with an absolute decrease in the mastectomy rate of 16.6% (95% CI 15.1–18.1%) [9]. In fact, this 16.6% reduction in the mastectomy rate was an underestimation of the potential benefit of NACT, as many of the patients were candidates for BCS at presentation and, with regard to the surgical approach, could not benefit from NACT. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 trial [10] and the European Organisation for Research and Treatment of Cancer (EORTC) 10901 trial [13], the rates of BCS after four cycles of anthracycline-based NACT in patients deemed to have required mastectomy if surgery had been the initial treatment were 27% and 23%, respectively. Paradoxically, although rates of pCR to NACT have increased markedly with the use of newer therapeutic agents and targeted therapies, rates of BCS have not risen. For example, in the NSABP B-27 trial [14], the addition of docetaxel to doxorubicin and cyclophosphamide NACT increased the pCR rate from 13.7% to 26.1% ($P < 0.001$), but the rates of BCS were not significantly different between the patients who received docetaxel and those who did not (61.6% vs. 63.7%; $P = 0.33$). More recently, in the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) trial in patients with HER2-overexpressing tumours [15], which compared chemotherapy plus trastuzumab and lapatinib with chemotherapy plus either lapatinib or trastuzumab, rates of pCR differed considerably: 51.3% with dual HER2 blockade, 29.5% with trastuzumab, and 24.7% with lapatinib. However, in patients who were not candidates for BCS at randomization, rates of BCS after NACT were 26.4% in the trastuzumab–lapatinib combination group, 27.7% in the trastuzumab group, and 26.4% in the lapatinib group [15]. Indeed, failure to translate increased pCR rates into a higher rate of BCS has been observed in multiple studies (Tables 28.1 and 28.2). This trend is somewhat inexplicable, but is probably attributable to the difficulty in evaluating the extent of residual disease after NACT and before surgery and confusion regarding whether resection of the entire volume of breast tissue originally occupied by the tumour is necessary. Additionally, some definitions of pCR include patients with residual ductal carcinoma in situ (DCIS), which can preclude BCS. Furthermore, just as patients who are candidates for primary BCS often opt for mastectomy [16], patient preference after NACT might also contribute to the observed rates of mastectomy in this setting.

28.1.3 Patient Selection for NACT to Enable BCS

Both anatomical and biological factors are useful in selecting patients with breast cancer in whom NACT is likely to result in tumour downstaging that enables BCS. For instance, patients with high-grade breast tumours that are oestrogen receptor (ER)-negative and/or HER2-positive have a higher likelihood of pCR to NACT. In one study, patients with ER-positive, HER2-negative luminal tumours, which are generally low grade, had a 6% pCR rate with paclitaxel, 5-fluorouracil, doxorubicin, and cyclophosphamide NACT, compared with 45% for HER2-positive or basal-like tumours (which are mostly negative for ER, progesterone receptor [PR], and HER2 and triple-negative breast cancer [TNBC]) [17]. In patients with ER-positive tumours, a 21-gene assay for estimation of disease recurrence (Oncotype DX©, Genomic Health, USA) is predictive of the probability of pCR to NACT, just as this assay is predictive of a benefit from chemotherapy added to endocrine therapy in the adjuvant setting [18]. The suitability of patients with infiltrating lobular carcinoma (ILC) for preoperative therapy to downstage tumours to enable BCS is uncertain. A meta-analysis of data from 12,645 patients with infiltrating ductal cancers and 1764 with ILC reported a pooled pCR rate for ductal cancers of 16.7% (95% CI 13.5–20.5) compared with 5.9% (95% CI 3.6–9.4%) for ILCs—a pooled odds ratio (OR) of 3.1 ($P < 0.00001$) [19]. In the 13 studies included in this meta-analysis that reported rates of BCS, a higher rate was observed in patients with ductal versus lobular cancers (54.8% vs. 35.4%; pooled OR 2.1; $P < 0.00001$). Of note, a comparison of patients with lobular cancer ($n = 75$) and those with ductal cancer ($n = 671$) in two prospective NACT trials found that, after adjusting for hormone-receptor status, HER2 status, histological grade, and p53 expression, rates of pCR did not differ between ductal and lobular cancers, indicating that these additional clinicopathological features could potentially be used to select the subset of patients with lobular carcinoma most likely to benefit from NACT [20]. Importantly, pCR is not absolutely necessary for BCS: only sufficient tumour shrinkage to enable resection of the tumour to clear margins with an acceptable cosmetic result is required. Nevertheless, patients who achieve a pCR are by definition candidates for BCS, and rates of pCR provide a minimum estimate of the proportion of patients likely to benefit from the NACT approach. On the basis of the current data, the patients in whom NACT is most likely to result in tumour downstaging to enable BCS are those with unicentric,

Table 28.1 Comparison of neoadjuvant chemotherapy regimens regarding their outcome in terms of pathological complete response and breast-conserving surgery rates: neoadjuvant trials and trials comparing preoperative versus postoperative administration

Trial	Preoperative therapy	*n*	ypT0/Tis ypN0 (%)	BCS (%)
ypT or *N*, pathological tumour or node category after chemotherapy; *BCS* breast-conserving surgery; *dd* dose dense; *A* doxorubicin; *Doc* docetaxel; *Tam* tamoxifen; *C* cyclophosphamide; *TAC* docetaxel–doxorubicin–cyclophosphamide; *n.a.* not available; *N* vinorelbine; *X* capecitabine; *E* epirubicin; *HER* human epidermal growth factor receptor; *CHT* chemotherapy; *H* trastuzumab; *AGO* Arbeitsgemeinschaft für Gynäkologische Onkologie; *Pac* paclitaxel; *PREPARE* Preoperative Epirubicin Paclitaxel Aranesp Study; *CMF* cyclophosphamide–methotrexate–5-fluorouracil; *SWOG* Southwest Oncology Group; *MDACC* MD Anderson Cancer Center; *FAC* 5-fluorouracil–doxorubicin–cyclophosphamide; *CALGB* Cancer and Leukemia Group B; *Bev* bevacizumab; *Cb* carboplatin; *NSABP* National Surgical Adjuvant Breast and Bowel Project; *ABCSG* Austrian Breast and Colorectal Cancer Study Group; *bpCR* breast pathological complete response; *EORTC* European Organization for Research and Treatment of Cancer; *FEC* 5-fluorouracil–epirubicin–cyclophosphamide				
GeparDo	dd A Doc × 4	126	9.5	69
	dd A Doc × 4 + Tam	122	5.7	69
GeparDuo	dd A Doc + Tam	453	10.2	66
	A C × 4 then Doc + Tam	454	19.2	75
GeparTrio pilot	TAC × 6	252	19.0	n. a.
	TAC × 2 then 4 × N X	33	6	n.a.
GeparTrio	TAC × 6	1085	18.7	68
	TAC × 8	686	29.0	69 responders
				57 nonresponders
GeparQuattro	E C × 4 then Doc + H +/– X	445	40	60
HER2 negative	E C × 4 then Doc × 4	343	18.7	68[a]
	E C × 4 then Doc + X × 4	345	16.5	67
	E C × 4 then Doc × 4 then X × 4	362	19.1	64
HER2 positive	CHT + H for HER2 positive	445	41.3	
AGO-1	E Pac × 4	335	6.6	58
	dd E × 3 then dd Pac × 4	333	13.2	
PREPARE	E C × 4 then Pac × 4	370	14.6	67
	dd E × 3 then dd Pac × 3 then CMF × 3	363	20.4	65
SWOG 0012	A C × 5 every 3 weeks then Pac × 12	179	20.7	n.a.
	A × 15 weekly + C daily then Pac × 12	177	24.3	
MDACC	FAC × 4	100	9.0	n.a
	dd FAC × 4	99	13	
CALGB 40603	Pac × 12 then dd A C × 4	108	39.0	n.a.
	+ Bev × 9 every 2 weeks	110	43.0	
	+ Cb × 6 every 3 weeks	113	49.0	
	+ Cb + Bev	112	60 0	
Older trials comparing pre-op				
and post-op administration				
NSABP B-18	A C × 4	747		67
	Primary surgery	759		60
ABCSG-07	CMF × 3	203	5.9 bpCR	66
	Primary surgery	195		60
EORTC 10902	FEC × 4	350	4.0	35
	Primary surgery	348		22

high-grade, ER-negative, and/or HER2-positive breast cancer [21, 22].

Multiple studies have evaluated the accuracy of MRI compared with physical examination, mammography, and ultrasonography in determining the presence and extent of viable tumour within the breast after NACT [23–27]. In a multi-institutional study of 41 women with palpable breast cancers, Yeh et al. [27] demonstrated that preoperative MRI had the best correlation with surgical specimen pathology when compared with physical examination, mammography, and ultrasonography. Furthermore, in 216 women who participated in the prospective, multi-institutional I-SPY trial [23], MRI was shown to be a better predictor of pathological response to NACT than clinical examination. A meta-analysis of 44 studies including a total of 2050 patients who received NACT found that the median sensitivity of MRI for the detection of residual cancer across studies was 0.92 and the median specificity was 0.60 [24]; however, accuracy differed

Table 28.2 Comparison of neoadjuvant chemotherapy regimens regarding their outcome in terms of pathological complete response and breast-conserving surgery rates: targeted therapy trials

Trial	Preoperative therapy	*n*	ypT0/Tis ypN0 (%)	BCS (%)
ypT or *N* pathological tumour or node category after chemotherapy; *BCS* breast-conserving surgery; *Pac* paclitaxel; *FEC* 5-fluorouracil–epirubicin–cyclophosphamide; *H* trastuzumab; *NSABP* National Surgical Adjuvant Breast and Bowel Project; *A* doxorubicin; *C* cyclophosphamide; *n.a.* not available; *L* lapatinib; *CHER-LOB* Chemotherapy, Herceptin, and Lapatinib in Operable Breast cancer; *NOAH* NeOAdjuvant Herceptin; *CMF* cyclophosphamide–methotrexate–5-fluorouracil; *HER* human epidermal growth factor receptor; *NeoALTTO* Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization; *P* pertuzumab; *Doc* docetaxel; *Cb* carboplatin, *TECHNO* Taxol Epirubicin Cyclophosphamide Herceptin Neoadjuvant; *E* epirubicin; *Bev* bevacizumab; *X* capecitabine; *Gem* gemcitabine				
Buzdar et al.	Pac × 4 then FEC × 4	19	26	53
	Pac × 4 then FEC × 4 + H × 24 weekly	23	65	57
		(164 planned)		
NSABP B-41	A C × 4 then Pac × 12			
	+ H weekly	177	49.4	n.a.
	+ L	171	47.4	n.a.
	+ H weekly + L	171	60.2	n.a.
CHER-LOB	Pac × 12 then FEC × 4			
	+ H weekly	36	25	67
	+ L	39	26	58
	+ H + L	46	47	69
NOAH	A + Pac × 3 then Pac × 4 then CMF × 3			
	HER2 negative	99	16	n.a.
	HER2 positive	118	19.0	13
	HER2 positive + H × 11 every 3 weeks	117	38.0	23
NeoALTTO	6 weeks L then 12 × Pac + L	154	24.7	43
	6 weeks H then 12 × Pac + H	149	29.5	39
	6 weeks L + H then 12 × P + H + L	152	51.3	41
TRYPHAENA	FEC + H + P × 3 then Doc + H + P × 3	73	56	n.a.
	FEC × 3 then Doc + H + P × 3	75	55	n.a.
	Doc + Cb + H + P × 6	77	64	n.a.
NeoSphere	Doc × 4 + H every 3 weeks	107	21.5	n.a.
	Doc × 4 + H + P every 3 weeks	107	39.3	n.a.
	H + P every 3 weeks	107	11.2	n.a.
	Doc + P every 3 weeks	96	18	n.a.
TECHNO	E C × 4 then Pac + H × 4	217	39.0	64
GeparQuinto				
HER2 positive	E C × 4 then Doc × 4 + H	309	44 6	64
	E C × 4 then Doc × 4 + L	311	30 2	59
HER2 negative	E C × 4 then Doc × 4 + Bev	956	21.7	62
	E C × 4 then Doc × 4	969	18.3	62
NSABP B-40	Doc × 4 then A C × 4	392	25.8	46
	Doc × 4 + X then A C × 4	393	23.2	43
	Doc × 4 + Gem then AC × 4	390	26.9	50
	Bev × 6 for half of all patients		23.0 with Bev	n.a.
			27.6 no Bev	

depending on the definition of pCR used and was lower in studies that permitted residual DCIS in the definition of pCR [24]. This meta-analysis also provided evidence that mammography had lower accuracy for detection of residual disease than MRI (relative diagnostic OR 0.27; 95% CI 0.07–1.02; $P = 0.02$), but differences in accuracy between MRI and ultrasonography and MRI and physical examination were not statistically significant [24]. All of these methods of evaluation are limited in their ability to detect scattered microscopic foci of viable carcinoma, which might have an impact on the success of BCS [26]. Current evidence indicates that the accuracy of MRI after NACT varies with ER, PR, and HER2 status and is greatest in patients with HER2-positive disease or TNBC, probably owing to the higher rates of pCR in these patients than those with other tumour types [28, 29]. Studies addressing the ability of MRI to identify patients who are appropriate candidates for BCS, as opposed to those aimed at identifying pCR or correlating tumour size

based on MRI assessment with pathological tumour size post NACT, are more limited. Straver et al. [30] examined pre-NACT and post-NACT MRI exams in 208 patients; in 35 patients (17%), MRI underestimated tumour size by more than 2 cm, which would have led to inappropriate attempts at BCS in 27 patients (13%). Conversely, MRI overestimated the extent of disease in nine patients (4%), leading to unnecessary mastectomy. Thus, the overall accuracy of MRI for the selection of surgical therapy was 83% [30]. In a study that investigated the relationship between MRI estimation of tumour size after NACT and positive surgical margins in 182 patients with breast cancer, one-third of patients (33%) in whom tumour size was underestimated by more than 2 cm had positive margins compared with 12% of those with lesser degrees of underestimation or overestimation of tumour size (P = 0.005); however, underestimation of tumour size by greater than 2 cm occurred in only 10% of patients [31]. In aggregate, the literature indicates that MRI is useful for selecting patients who are candidates for BCS after NACT. In patients with malignant calcifications, a post-NACT mammogram is also useful for planning the extent of the resection: although calcifications do not always indicate residual malignancy [32], the presence of residual disease cannot be reliably excluded unless all radiographic abnormalities are removed.

28.1.4 Surgical Issues

In patients undergoing NACT with the potential for breast tumour downstaging to enable BCS, the tumour site should be marked with a clip before initiating NACT. Resection of the entire volume of breast tissue originally occupied by tumour is not necessary [33]; however, no consensus has been reached on what constitutes an adequate surgical margin in this setting. The NSABP B-18 trial [34] used the standard NSABP margin definition of no ink on tumour and, after controlling for age and tumour size, found no statistically significant differences in local recurrence between patients who required NACT for downstaging to BCS candidacy, those who were candidates for BCS before NACT, and those who underwent BCS and received adjuvant therapy. Similarly, the meta-analysis by Mieog et al. [9] reported no significant differences in local recurrence for patients with breast cancer who received NACT versus those who received adjuvant therapy, including the subset of patients requiring NACT to downstage the primary tumour to enable BCS. Thus, BCS after NACT can clearly be safe, although the 'Swiss cheese' pattern of response, characterized by scattered microscopic foci of residual viable tumour, has been shown to predict an increased risk of local recurrence in a large population of patients with breast cancer treated with NACT at the University of Texas MD Anderson Cancer Center (MDACC) [35]. In our opinion, the presence of multiple scattered tumour foci in close proximity to the surgical margin warrants consideration of re-excision when less than the original pretreatment tumour volume has been resected after NACT. In the absence of this pattern of tumour response, a margin of no ink on tumour is probably adequate.

28.1.4.1 Targeted Breast Surgery

Breast-conserving therapy (BCT) consisting of surgical removal of the primary tumour followed by whole breast irradiation is an alternative to mastectomy which results in equivalent long-term survival [36]. Although rates of BCT have increased over time worldwide, there remains remarkably little consensus about what amount of normal breast tissue should be removed as a margin to minimize the risk of local recurrence. The conclusion of the SSO (Society of Surgical Oncology)–ASTRO (American Society for Radiation Oncology) Consensus Panel reinforced the importance of obtaining negative margins defined as no ink on tumour (invasive cancer or DCIS) to optimize local control [37]. The most important and potentially practice-changing conclusion was based on the finding in the meta-analysis of Houssami et al. that margins of 1, 2, or 5 mm were not associated with significantly different risks of local recurrences [38]. This meta-analysis could not be used to demonstrate whether a margin of no ink on tumour is adequate for patients with invasive lobular cancer, an EIC in association with invasive cancer, and tumours of unfavourable biological subtype (i.e., triple-negative breast cancer) and in young patients.

Oncoplastic principles were introduced into breast-conserving surgery 20 years ago to allow oncologically safe breast conservation, by performing a wide excision for larger or poorly located tumours, while limiting the risk of postoperative deformities [39]. Numerous surgical techniques with tissue displacement and tissue replacement have been published with different indications, incision lines, and suggested rotation techniques, missing a systematic and structured approach for oncoplastic breast surgery [40]. During the last years, we have defined five reconstruction principles introducing a new concept of breast-conserving surgery, termed *targeted* (*oncoplastic) breast surgery* [40–43].

We prospectively defined six major reconstruction principles in oncoplastic breast-conserving surgery (BCS) based on the localization, size of the segmental resection defect, size of the breast, and the necessity for skin resection during breast-conserving therapy. These major principles were BCS glandular rotation, BCS dermoglandular rotation, BCS thoracic wall advancement, BCS tumour-adapted reduction mammoplasty, BCS thoracoepigastric flap, and BCS latissimus dorsi flap (Figs. 28.1, 28.2, 28.3, 28.4, 28.5, 28.6, and 28.7). Partial mastectomy defects could be reconstructed

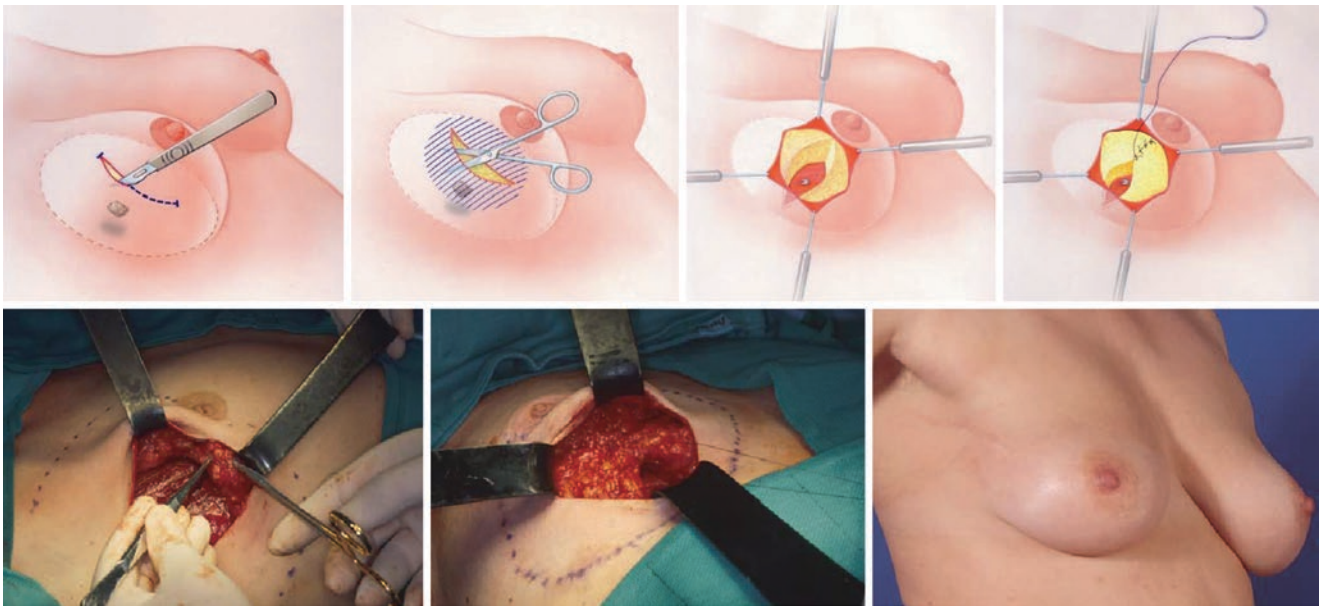

Fig. 28.1 Principles in targeted oncoplastic breast-conserving surgery: BCS glandular rotation. *BCS* breast-conserving surgery

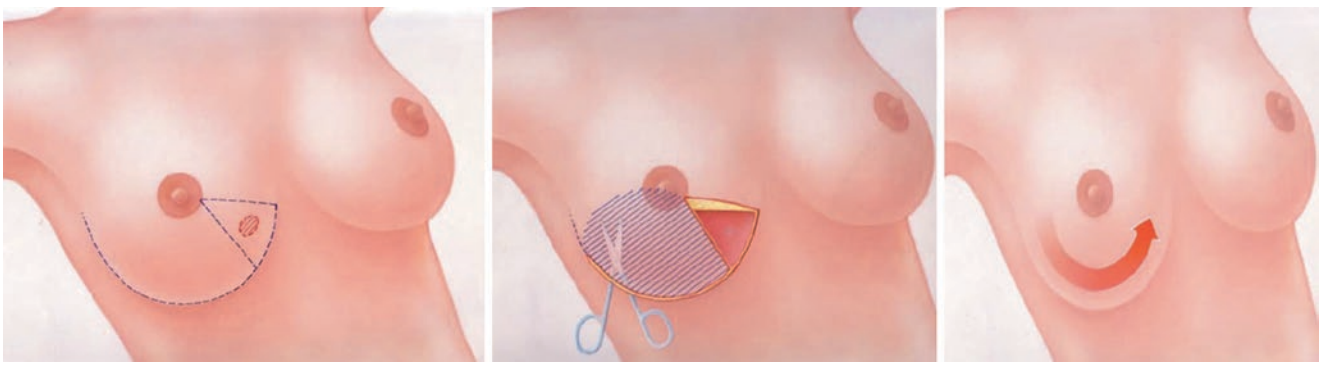

Fig. 28.2 Principles in targeted oncoplastic breast-conserving surgery: BCS dermoglandular rotation. *BCS* breast-conserving surgery

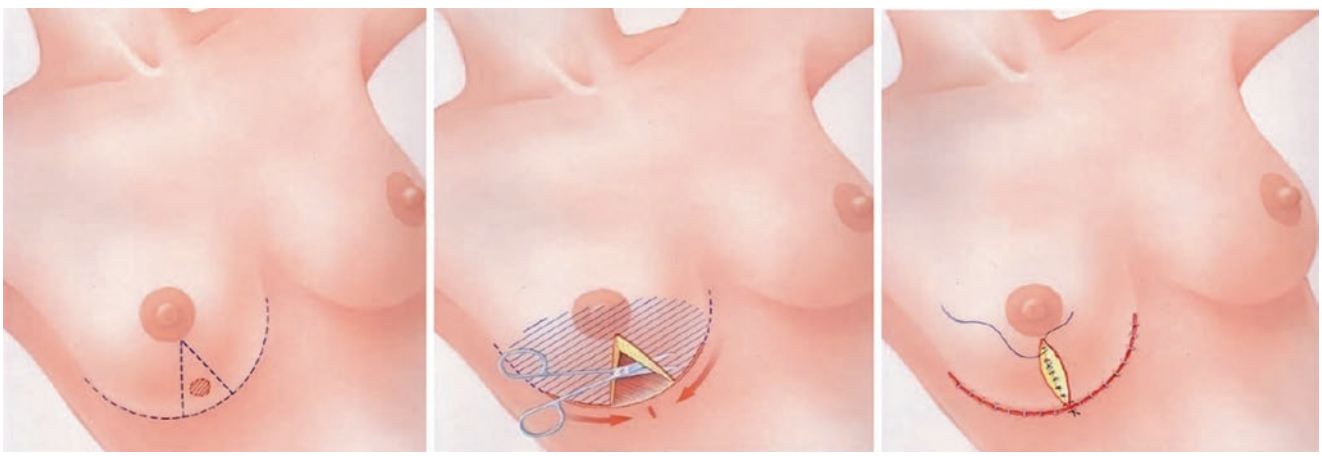

Fig. 28.3 Principles in targeted oncoplastic breast-conserving surgery: BCS dermoglandular rotation (tumour-adapted mastopexy). *BCS* breast-conserving surgery

Fig. 28.4 Principles in targeted oncoplastic breast-conserving surgery: BCS thoracic wall advancement according to Rezai. *BCS* breast-conserving surgery

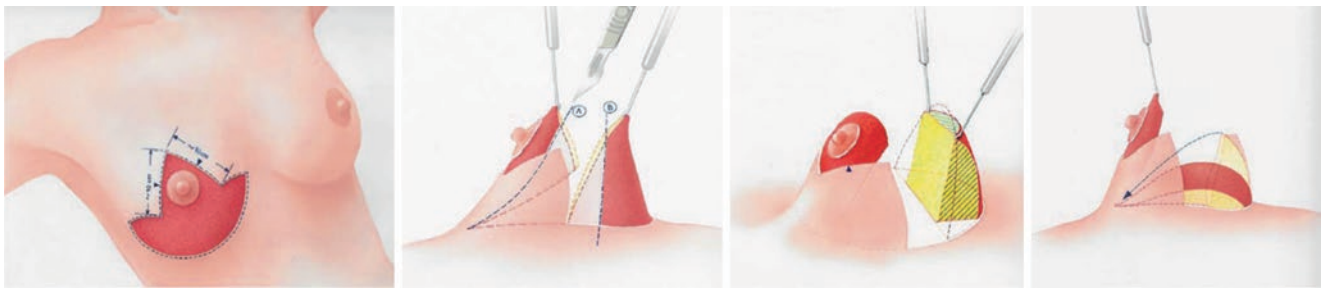

Fig. 28.5 Principles in targeted oncoplastic breast-conserving surgery: BCS tumour-adapted reduction mammoplasty according to Rezai. *BCS* breast-conserving surgery

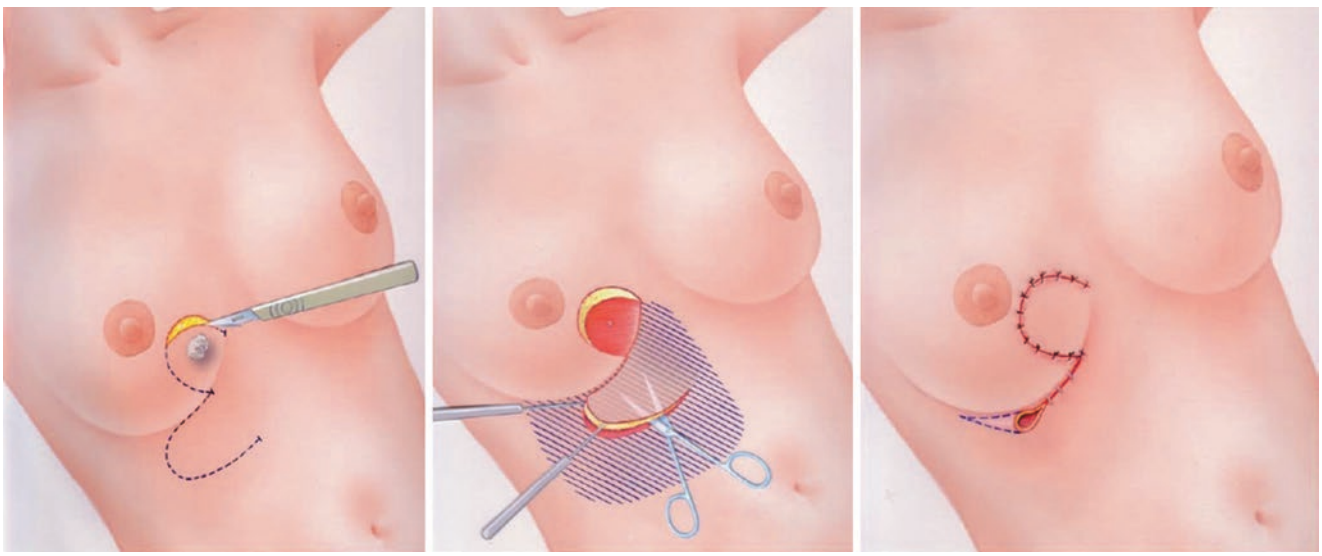

Fig. 28.6 Principles in targeted oncoplastic breast-conserving surgery: BCS thoracoepigastric flap. *BCS* breast-conserving surgery

during BCS with these five oncoplastic principles in 97%. The cosmetic results were good or excellent in 95%. A tumour-free resection margin of 1 mm was mandatory (according to German guidelines) and achieved in 91% during first surgery, while in 5% secondary mastectomy was required. Local recurrences were diagnosed in 1.9% with a median follow-up of 4.2 years.

Our understanding of breast cancer biology has advanced considerably since the initial trials comparing BCT and mastectomy more than 30 years ago. It is apparent that factors such as tumour biology and the availability of effective systemic treatment are at least as important as microscopic residual disease burden in determining local control of breast cancer. Adoption of no ink on tumour as

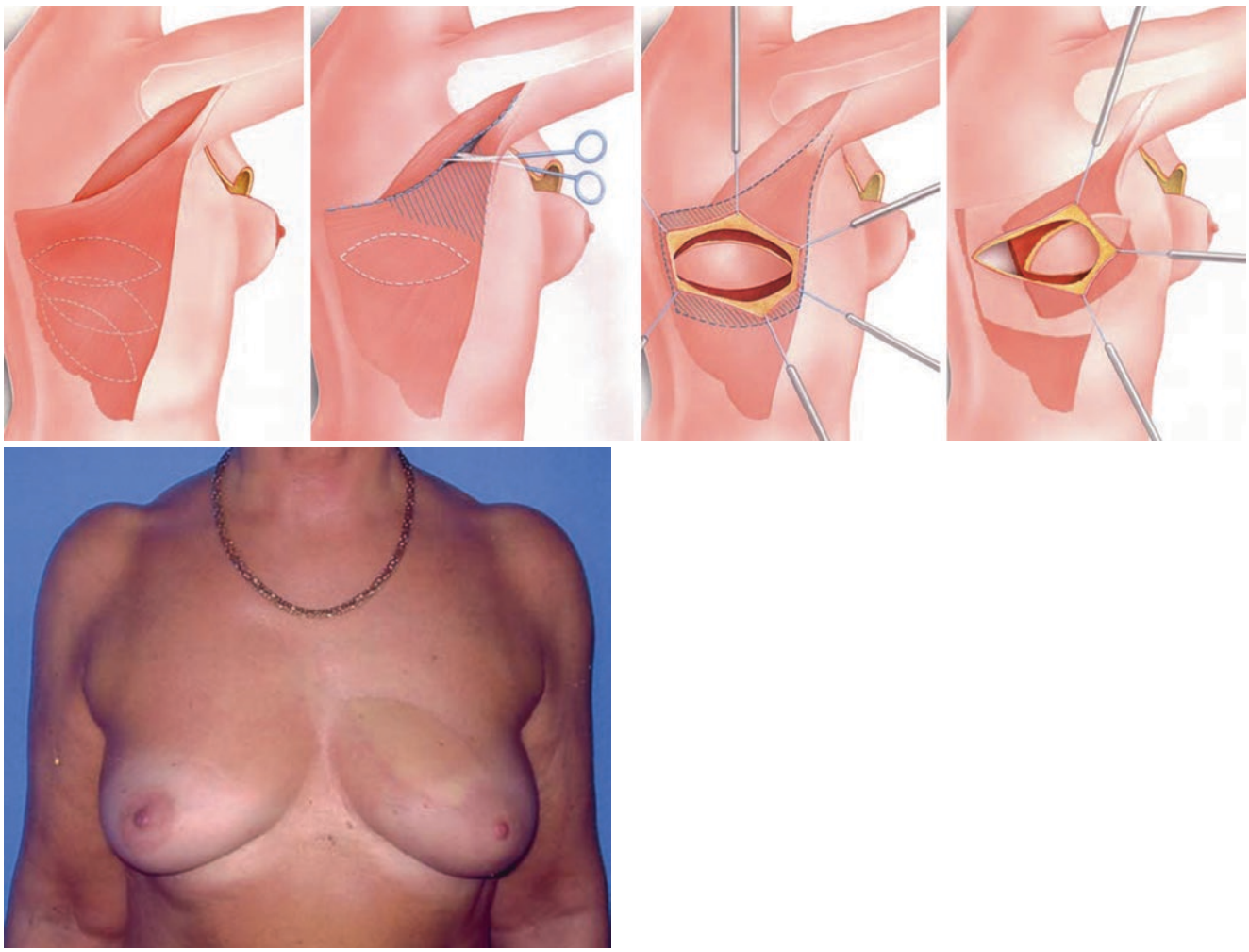

Fig. 28.7 Principles in targeted oncoplastic breast-conserving surgery: BCS latissimus dorsi flap. *BCS* breast-conserving surgery

the standard negative margin definition has clear potential to decrease the use of re-excision and large quadrantectomy-type resections. Adoption of a minimal margin definition removes the rational for the *old* concept of oncoplastic breast surgery—introduced 20 years ago. Further development of the traditional concept of oncoplastic breast surgery to a concept of *targeted* (*oncoplastic*) *breast surgery* with five defined oncoplastic principles allows the reconstruction of segmental resection defects during breast-conserving therapy with highest clinical applicability and results in favourable oncological and aesthetic outcomes. This approach might be useful in extending the indications for breast-conserving therapy. The adoption of a minimal margin definition does not remove the rational for a *new* concept of targeted oncoplastic breast surgery. Targeted oncoplastic breast surgery depends on the anatomical, pathological, and reconstructive aspects of breast cancer to achieve favourable local outcomes for the patients—combining oncological and aesthetic prerequisites [44].

28.1.4.2 Surgical Complications Following NACT

An aspect of NACT that has not yet been investigated thoroughly is the effect of preoperative treatment on surgical complications. The influence of new agents such as biologicals and dose-dense therapies on postoperative wound healing, wound infection, haematoma formation and the need for reoperation has still scarcely been studied. In a recent retrospective analysis [45], data were collected from 44,533 patients after breast surgery. A multivariable regression analysis was performed to identify predictors of postoperative wound complications; 2006 patients received NACT before surgery. Wound complication rates were generally low and comparable in the neoadjuvant treatment and primary surgery groups (3.4 vs. 3.1%). It was concluded that NACT does not influence postoperative wound healing, although there was a trend towards a higher rate of wound complications (4.0%) among patients who had mastectomy and immediate reconstruction after NACT. However, these rates may be an underestimate as postoperative complications

requiring reoperation were excluded. It is understandable that mastectomies with immediate or delayed reconstruction have higher postoperative complication rates than BCS [46]. In smaller series [47–49] of immediate breast reconstruction following NACT, complication rates after mastectomy and immediate autologous or expander/implant reconstruction with or without preceding NACT were compared and reported to be similar. Bearing in mind the small sample sizes, NACT did not, however, seem to affect postoperative complication rates.

Some reports have raised doubt about whether the use of preoperative bevacizumab is safe [50]. Bevacizumab in addition to chemotherapy increases the pCR rate. The GeparQuinto study [51] reported a non-significant increase in overall surgical complications after preoperative addition of bevacizumab (11.0 vs. 15.3%; $P = 0.12$), but revealed an increased risk for patients who required two or more operations to achieve clear margins for BCS [52]. Golshan et al. [53] reported an increased complication rate when performing immediate breast reconstruction using expanders. In a single-arm study, with only 51 patients enrolled, which evaluated neoadjuvant cisplatinum plus bevacizumab, no significant increases in wound healing complications following BCS were observed compared with the results of a previous study in which cisplatinum was given without bevacizumab. Nevertheless, loss of the reconstruction (implant or expander) was reported in four of eight patients. A further study [54] reported no difference in overall surgical complication rate among patients treated with neoadjuvant doxorubicin–cyclophosphamide–paclitaxel with or without bevacizumab. Patients in the two cohorts undergoing mastectomy with or without reconstruction (autologous tissue or implant/expander) were compared. Again, the rate of complications was higher when implants/expanders were used for immediate reconstruction following administration of bevacizumab in a cohort of 119 patients.

28.1.5 Locoregional Recurrence After NACT

In a meta-analysis [55] of nine randomized clinical trials, the clinical outcome of 3861 patients receiving the same systemic therapy either before or after surgery was compared. No significant difference in cancer-related death, disease progression, or distant disease recurrence was reported. A significant increase in LRR rate was observed in the neoadjuvant treatment arm (relative risk 1.22; $P = 0.015$). Four of the nine studies included in this meta-analysis allowed RT alone, without any breast surgery, when a complete clinical response was achieved. The NACT regimens administered in those studies are not comparable with those of the current standard of care, and clinical response was assessed by palpation and X-ray mammography. In addition, complete response was not proven histologically by biopsy before the decision to omit surgery was taken. Thus, an increase in LRR in the neoadjuvant arm is understandable.

Long-term follow-up results of the NSABP B-18 and B-27 trials have been published. These two studies included a total of 3088 patients undergoing NACT or adjuvant chemotherapy. All underwent surgery in the course of treatment. RT was limited to WBI following BCS. Chest wall RT following mastectomy or RT of regional lymph nodes was not allowed in the trial protocols, so an influence of unstandardized RT on locoregional control was avoided. The 10-year cumulative LRR rate after NACT was 12.3% for patients who had a mastectomy and 10.3% for those treated with BCS and consecutive WBI. Clinical tumour size greater than 5 cm in patients who had a mastectomy and age below 50 years in the BCS group had a significant impact on the risk of LRR by 10 years. Clinically node-positive (cN+) disease before NACT and pathological nodal involvement after NACT were independent predictors of LRR, irrespective of type of surgical therapy. Patients who failed to achieve downstaging of the axilla (cN + to ypN0) and breast pCR were at higher risk of LRR. Unfortunately, data concerning hormone receptor and HER2 status were not available, and it could not therefore be determined whether certain subgroups may benefit more or may be at increased risk of LRR after NACT. Moreover, the direct comparison of LRR rates between the two groups in NSABP B-18, which received the same type of chemotherapy (one group before and one after surgery), was not reported.

If subgroups at increased risk of LRR could be identified, this knowledge could be included when deciding on surgical treatment. In a recent meta-analysis [55] of 12,592 patients with breast cancer treated with initial surgery (BCS or mastectomy), it was stated that the risk of LRR may vary between tumour subtypes. Patients with triple-negative breast cancer or a HER2-positive phenotype have a higher risk of LRR than patients with luminal tumours. Lowery et al. [56] reported a LRR rate of 7.1% for BCS and 9.0% for mastectomy at a median follow-up of 57 months for patients with HER2-positive breast cancer, these patients showing the highest risk of LRR. Keeping in mind that these data were collected before the era of trastuzumab and that all NACT was excluded, these rates may not apply to modern NACT regimens. All patients who had BCS underwent adjuvant RT, and 44% of those having a mastectomy received chest wall RT. Adjuvant chemotherapy was administered to 48% of all patients.

Young age is also a risk factor for increased risk of local recurrence. However, it seems that this is especially true for young patients without a pCR. In one study [57], of women who did not achieve a pCR, the LRR rate among those aged 35 years or less was significantly higher than that among women aged 36–50 years ($P = 0.024$). However, there was no age-related difference among women who achieved a pCR.

Is it possible that microscopic residual tumour is left behind when BCS is performed within new margins? It could be speculated that such resistant residual tumour could increase the overall risk of LRR. The main target of NACT is shifting from merely downstaging to monitoring tumour response and tailoring therapy and predicting clinical outcome. At the San Antonio Breast Cancer Symposium 2011, the German Breast Group presented data from a meta-analysis of seven prospective neoadjuvant trials with a total of 6377 patients. LRR rates were analysed according to initial tumour stage, intrinsic tumour subtype, type of surgery, pCR rate, and nodal status. At a median follow-up of 46.2 months, 485 patients had experienced LRR. LRR rates for BCS were significantly lower than those for mastectomy. Not surprisingly, the percentage of women undergoing BCS declined with increasing initial clinical tumour (cT) category (ranging from 77.7% for cT1 to 19.1% for cT4d), and LRR rate rose with increasing tumour size after NACT (from 4.7% for ypT0 to 31.2% for ypT4d). The LRR rate was higher among patients with non-invasive residual disease (9.9 vs. 3.7%). Comparing tumour subtypes, despite achieving a pCR, luminal B/HER2-positive tumours had a higher LRR rate (8.1%) than all other subtypes. Among patients who did not achieve a pCR, triple-negative and non-luminal-like HER2-positive tumours both displayed an extraordinary LRR rate of about 18%.

Weksberg et al. [58] investigated the prognostic outcome of salvage therapy in patients with local recurrence after NACT and BCS. Data were analysed retrospectively for 1589 patients, of whom 448 had undergone surgery after NACT. Among these, 2.6% of patients initially treated with BCS and 5.8% treated with NACT and subsequent BCS experienced LRR at a median follow-up of 91 months. Higher nuclear grade, higher tumour stage, and larger number of involved lymph nodes in the NACT group may account for the difference in LRR rate itself. No significant differences in DFS, OS, and locoregional control were detected in the two groups following salvage treatment for isolated LRR.

Therefore, resection within new margins after NACT is safe and should be offered to more patients, enabling translation of the increasing pCR rates into higher BCS rates and avoidance of unnecessary mastectomies.

28.1.6 Management of the Axilla After NACT

The use and timing of sentinel lymph node biopsy (SLNB) in patients who have undergone NACT has been the subject of considerable debate. Initial concerns regarding the feasibility and accuracy of SLNB following chemotherapy were centred on the potential for altered lymphatic drainage as a result of lymphatic tissue fibrosis or vessel blockage by tumour emboli, as well as the possibility that the effects of chemotherapy might not be uniform throughout the nodal basin. Opponents of SLNB after NACT also argued that knowledge of the axillary node status before NACT was necessary to identify optimal candidates for adjuvant radiotherapy. For women presenting with clinically node-negative disease, these concerns have largely been addressed, and SLNB after NACT is now accepted as standard care [59]. More recent controversy has surrounded the use of SLNB after chemotherapy in patients who present with clinically positive needle biopsy-proven nodal metastases.

28.1.6.1 Clinically Node-Negative Disease

Numerous studies, including the NSABP B-27 trial [60], a large single-institution series from the MDACC [61], and several meta-analyses [62, 63] have established that sentinel lymph node (SLN) identification rates and false-negative rates after NACT are comparable to those reported in patients with breast cancer who undergo upfront surgery. In the MDACC experience, SLN identification rates were 97.4% for women who underwent SNLB after NACT ($n = 575$) and 98.7% for patients treated with upfront surgery ($n = 3, 171$; $P = 0.017$), and false-negative rates were similar: 5.9% versus 4.1% ($P = 0.39$). After a median follow-up duration of 47 months, regional disease recurrence had occurred in 0.9% of the patients who underwent upfront surgery and SLNB compared with 1.2% in the NACT group—a statistically insignificant difference. This study also demonstrated that NACT could be used to downstage disease in the axilla in patients presenting with clinically node-negative T2 and T3 breast tumours, resulting in fewer axillary node dissections without compromising locoregional control: SLN-positive rates compared with upfront surgery were 20.5% versus 36.5% ($P < 0.0001$) and 30.4% versus 51.4% ($P = 0.04$) for women with T2 and T3 tumours, respectively. These data are consistent with those from NSABP B-18 [10], a randomized trial of preoperative versus postoperative chemotherapy, which showed that patients who received preoperative chemotherapy were more likely to have pathologically negative lymph nodes compared with those who underwent surgery first (58% vs. 42%; $P < 0.0001$), demonstrating that NACT can eradicate nonpalpable nodal disease in some patients.

Despite the proven ability of NACT to downstage disease in the axilla, the relative importance of pretreatment nodal stage versus postchemotherapy nodal stage on locoregional recurrence (LRR) risk and the need for adjuvant radiotherapy remain uncertain. Updated data from a combined analysis of NSABP B-18 and B-27 [64], trials of NACT in patients with operable breast cancer that did not allow regional nodal radiotherapy and/or radiotherapy to the chest wall (radiation treatment of the breast was performed in patients who underwent lumpectomy), have provided important information regarding predictors of LRR in this setting. In both trials [10, 60], approximately 70% of patients treated in the NACT

groups were clinically node negative before treatment. At 10 years of follow-up in 3,088 patients who received NACT in these trials, LRR events had occurred in 335 (10.9%) [65]; patient age, clinical tumour size, clinical nodal status before NACT, and pathological nodal status and breast tumour response after NACT were independent overall predictors of LRR. Importantly, among the clinically node-negative patients treated with lumpectomy and breast radiotherapy after NACT, rates of regional nodal recurrence were low (0.5–2.3%) and were not influenced by pathological node status nor pathological breast tumour response. Among clinically node-negative patients treated with NACT followed by mastectomy, regional nodal recurrence rates were also low, irrespective of tumour size (2.3–6.2%); however, rates of chest wall recurrence were greater in patients with clinically negative nodes but pathologically node-positive disease and were negatively correlated with breast tumour response.

Taken together, these data demonstrate that SLNB after NACT in patients with clinically node-negative disease is feasible and accurate and that NACT decreases the number of patients with a positive SLN, thereby sparing patients the morbidity of ALND, without compromising subsequent treatment recommendations or locoregional control. These findings also raise questions about current recommendations that all patients receiving NACT should undergo axillary ultrasonography with biopsy of abnormal nodes [59].

28.1.6.2 Clinically Node-Positive Disease

The success of SLNB after NACT in patients presenting with clinically node-negative disease, combined with increasing rates of pCR demonstrated in trials using modern chemotherapy regimens and targeted therapies, has led to increased interest in the use of SLNB after NACT in patients who present with clinically positive nodes. This issue is particularly relevant for patients with ER-negative and/or HER2-positive disease treated with preoperative anti-HER2 therapy, in whom pCR rates exceed 50% [66, 67]. Early evidence that this approach might be feasible came from the NSABP B-27 trial [60], which included patients with both clinically negative and positive nodes, although histological documentation of pathological nodal status was not required before NACT. After NACT, 428 of 2411 (18%) patients underwent attempted SLN identification and removal before the required ALND—23.8% of the 428 patients in whom SLN biopsy was attempted had clinically positive nodes before NACT. Among the 343 patients in whom both SLNB and ALND were performed successfully, the overall false-negative rate was 10.7% (15 of 140 node-positive patients had a negative SLNB), with no significant difference according to pretreatment nodal status (P = 0.51). Similarly, a report from a French prospective multicentre trial of SLNB after NACT found no significant difference in the false-negative rates between patients who were clinically node positive (n = 65) versus clinically node negative (n = 130) at presentation (15% vs. 9.4%; P = 0.66) [68]. These observations were not supported by smaller, single-institution case series of SLNB after NACT in patients for whom positive nodal status was documented with pretreatment biopsy [69]. The largest of these series, from the MDACC, included 150 patients with biopsy-proven nodal metastasis; 111 of these patients also underwent SLNB and ALND after NACT, and the SLN identification rate was 93% and the false-negative rate was 20.8%, leading to the conclusion that ALND remained the standard of care in this setting [70].

Three multicentre studies addressing the feasibility of SLN after NACT in patients with clinically node-positive disease have, however, challenged the conclusion that ALND is required for all clinically node-positive patients [71–73]. The Sentinel Neoadjuvant (SENTINA) trial [74], a four-arm prospective multicentre trial by the German Breast Group, included 1737 patients who all received at least six cycles of anthracycline-based NACT. All clinically node-negative patients had upfront SLNB; those who had pathologically negative SLNs had no further axillary node surgery (arm A; n = 662), and those who were SLN positive underwent a second SLNB and ALND after NACT (arm B; n = 360). Clinically node-positive patients (n = 715) underwent NACT; those who converted to clinically node-negative disease (as documented by physical examination and ultrasonography of the axilla) underwent SLNB and ALND (arm C; n = 592, pre-NACT nodal status confirmed in 149 [25%]), and the women who remained clinically node positive had ALND (arm D, n = 123). Re-operative SLNB (arm B) resulted in the lowest SLN identification rate (60.8%) and an exceedingly high false-negative rate (51.6%), clearly demonstrating that SLNB should not be performed both before and after chemotherapy. SLN identification rates were also lower than expected in arm C (80.1%) and were associated with a false-negative rate of 14.2%, although the false-negative rate was lower when three or more SLNs were removed (7.3%)—both end points were improved when SLN mapping was performed using the dual mapping technique (with radioisotope and blue dye).

The importance of SLNB technique in patients with needle biopsy-proven nodal involvement was also highlighted in the American College of Surgeons Oncology Group (ACOSOG) Z1071 trial [72], a phase II study that enrolled 756 women with T0–T4, biopsy-proven N1 or N2 disease; 663 patients had clinical N1 disease, 649 of whom completed NACT and subsequently underwent SLNB and ALND. Surgeons were encouraged to use the dual mapping technique for SLN identification and to remove at least two SLNs. The SLN identification rate was 92.9%, similar to the rate reported for SLNB after NACT in clinically node-negative patients and superior to those reported in the SENTINA trial; however, the overall false-negative rate of

12.6% was similar to the German Breast Group experience in arm C of the SENTINA study despite the fact that axillary lymph node response to NACT was not considered in selecting patients for SLNB. To be consistent with the accepted false-negative rate in patients presenting with clinically negative nodes, the prespecified criteria for success in the Z1071 trial were a false-negative rate of ≤10%; thus, the study did not meet this end point. However, as reported in the SENTINA trial, when three or more SLNs were removed, the false-negative rate was 9.1%, demonstrating that surgical technique is critical when considering SLNB in this setting and that routine imaging of the axilla post NACT might not be necessary. These findings in biopsy-proven clinically node-positive breast cancer have now also been reproduced in the smaller Sentinel Node Biopsy Following Neoadjuvant Chemotherapy (SN FNAC) study [71]. In this study, removal of one SLN was associated with a false-negative rate of 18.2%, and removal of more than two SLNs was associated with a false-negative rate of 4.9%.

The relationship between the number of SLNs removed and the false-negative rate of the procedure is not a new concept. Nearly all early prospective trials of SLN biopsy in patients with early-stage breast cancer documented the same effect: lower false-negative rates with increasing numbers of nodes removed [75–78]. However, one must also consider that the median number of SLNs removed in the SENTINA trial was 2 [71–73], as it was in NSABP B-32 and other large prospective trials of upfront SLN biopsy, suggesting that three or more SLNs cannot be identified in many patients. Indeed, in 2014, the AMAROS trial of radiotherapy versus surgery in patients with a positive SLN demonstrated that only 382 (27%) of the patients randomized had three or more SLNs identified in the setting of upfront SLN biopsy [79]. Similarly, among 641 clinically N1 patients who converted to clinically node-negative disease in the Z1071 trial and among 592 patients in arm C of the SENTINA trial, 57% and 34% of patients, respectively, had three or more SLNs removed [55, 56]. Therefore, substantial numbers of patients who convert from clinically node-positive to clinically node-negative disease after NACT are unlikely to have three or more SLNs identified after NACT and, as demonstrated in all three studies to date [71–73], omitting ALND in these patients might be associated with an unacceptably high false-negative rate. Of note, no data support random sampling of nearby axillary lymph nodes to replace SLN mapping and identification of at least three nodes following NACT; thus, surgeons will need to monitor their own performance in this regard, and until data on the clinical significance of leaving axillary lymph node disease behind after NACT are available, patients should be informed that ALND could be indicated if SLN mapping is unsatisfactory.

28.1.7 Significance of Extent of Residual Nodal Disease

The relevance of the distinction between post-NACT isolated tumour cells (ypN0i+, <0.2 mm), micrometastatic disease (ypN1mi, 0.2–2.0 mm), and macrometastatic disease (ypN+, >2.0 mm) in SLNs is another factor that remains unclear. In patients who have not received NACT, the size of the SLN metastasis is correlated with the likelihood of additional nodal disease, and low-volume SLN disease does not always mandate completion axillary node dissection [74, 80, 81]. By contrast, according to the 7th Edition of the American Joint Committee on Cancer (AJCC) staging system [82], patients treated with NACT who are ypN0i+ or ypN1mi at SLNB are considered to have residual nodal disease, and ALND remains the standard of care. In the SN FNAC study [71], SLN metastases of any size were considered positive, and no correlation between the size of the SLN metastases and the rate of positive non-SLNs was found; however, if ypN0i+ SLN disease was considered SLN negative, the false-negative rate of the procedure would have increased from 8.4% to 13.3%. The Z1071 trial investigators also reported on a subset of 470 patients who had at least two SLNs identified and for whom pathological information regarding the presence of micrometastatic disease in the SLN, identified by immunohistochemistry or haematoxylin and eosin staining, was available [83]. When micrometastatic disease was included in the definition of residual nodal disease after NACT, the pCR rate decreased from 36.0 to 33.8% and the false-negative rate decreased from 11.3 to 8.7%. As ALND was performed in all patients in the Z1071, SENTINA, and SN FNAC trials, these studies provide no information regarding the clinical significance of leaving disease behind after NACT. An important consideration is that the potentially chemoresistant disease that persists after NACT might not be associated with the same outcomes demonstrated in the NSABP B-32 and Z0011 trials of upfront surgery, in which both micrometastatic and macrometastatic diseases remaining in the axilla did not compromise locoregional control or survival [74, 80, 81].

Failure to identify residual nodal disease after NACT might also have important implications for decisions regarding radiotherapy. In the updated analysis of NSABP B-18 and B-27 trials, clinically node-positive patients who received NACT and remained pathologically node positive experienced the highest rates of LRR following ALND, ranging from 15 to 22% after lumpectomy and radiotherapy of the breast and from 17 to 22% after mastectomy [65], implying that both groups should be considered for adjuvant radiotherapy: regional nodal radiotherapy in addition to breast radiotherapy for those who undergo BCS and chest wall radiotherapy for those treated with mastectomy. The question of whether completion ALND can be omitted in

favour of axillary radiotherapy in patients with positive SLNs after NACT is being addressed in the ongoing phase III A011202 trial, conducted by the Alliance for Clinical Trials in Oncology [84]. By contrast, in the NSABP trials, patients with clinically node-positive disease who had a pCR at mastectomy (ypT0N0) experienced excellent locoregional control (0% LRR at 10 years) [65], suggesting that response to NACT can be used to select patients who do not need postmastectomy radiotherapy. This concept is currently being tested in the NSABP B-51/Radiation Therapy Oncology Group (RTOG) 1304 (NRG 9353) trial, a phase III randomized trial of more versus less radiotherapy in women with clinically node-positive breast cancer who become pathologically node negative after NACT [85]. If additional radiotherapy in this setting does not affect the risk of LRR, NACT could become the new standard to facilitate a tailored approach to locoregional therapy in patients with operable node-positive breast cancer. For patients who remain node positive following NACT, accurate detection of residual disease is equally important, as these patients could potentially have some level of resistance to systemic therapy and, therefore, might be candidates for future trials of novel agents.

28.1.8 Conclusions

The use of NACT for the treatment of patients with breast cancer reduces the need for mastectomy and axillary dissection, decreasing the morbidity of surgery, without increasing the risk of LRR. Hormone receptor status and HER2 status can be used to select the patients most likely to experience a pCR with NACT. However, increasing rates of pCR with contemporary therapeutic agents (such as HER2-targeted therapies) have not been accompanied by a parallel increase in rates of BCS. Future trials of NACT should examine whether this pattern reflects an inability to accurately assess the extent of residual disease preoperatively or surgeon or patient preference. Improved understanding of the optimal negative margin width for BCS after NACT and the adoption of *targeted breast surgery* could also increase rates of BCS.

In patients with breast cancer who are clinically node negative at presentation, NACT often results in downstaging of axillary disease; SLNB after NACT provides an accurate indication of axillary lymph node involvement in this setting and can, therefore, guide the use of completion ALND, and this approach is associated with a low rate of LRR. The management of patients who are clinically node positive at presentation is in evolution—recent trials suggest SLNB is accurate if three or more sentinel nodes are obtained, but outcome data from patients treated with SLNB alone in this setting are lacking. Although false-negative rates for SLNB after upfront surgery of 10% are associated with a risk of LRR of <1%, whether this holds true for the potentially drug-resistant disease left behind after NACT remains unclear.

One of the great opportunities provided by NACT is the ability to tailor the extent of locoregional therapy based on the preoperative treatment response. The appropriate therapy will probably vary not only by response but also by ER, PR, and HER2 status; the failure to achieve pCR in patients with tumours that lack ER, PR, and HER2 could be indicative of a much higher risk of LRR than in patients with ER-positive tumours who receive at least 5 years of endocrine therapy or patients with HER2-positive disease who are treated with complete anti-HER2 therapy after NACT. Ongoing clinical trials will help to address these issues and to define the relative importance of pretreatment and posttreatment stage on the risk of locoregional recurrence.

The concept of *targeted breast surgery,* including five principles for breast-conserving surgery after NACT, is a recommended concept of surgical techniques optimizing local control and aesthetic outcome for patients—initially developed for primary BCS. Targeted breast surgery is a further development of the classical concept of oncoplastic breast surgery with wide local resection based on the new minimal resection margin width definition (*no ink on tumour*).

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Surgical Treatment of the Primary Tumor in Patients with Metastatic Breast Cancer (Stage IV Disease)

29

Mattia Intra

The widespread uptake of breast cancer screening, together with heightened population awareness, means that most breast cancers in the Western world are detected at an early stage. Recent tumor registry studies from the United States and Europe have shown that metastatic breast cancer (BC) accounts for 4–5% of all cases [1–3]. However, in developing nations, the proportion of patients with metastatic BC at diagnosis is greater, ranging from 10% in Malaysia [4] to 24% in Nepal [5] and 44% in Nigerian women [6]. In early breast cancer, high-quality evidence from randomized controlled trials and meta-analyses is available to support the majority of treatments we perform. In comparison, there is a lack of level I evidence and accepted standard-of-care therapies available for patients with metastatic BC.

Metastatic BC is considered to be a fatal disease, regardless of whether distant metastases are discovered at initial presentation (de novo stage IV) or following apparently successful therapy of localized disease and an intervening disease-free interval (metachronous stage IV). As stage IV disease is considered incurable, the goals of treatment are only the prolongation of life and the palliation or prevention of symptoms. In both the situations de novo stage IV and metachronous stage IV, the primary and most important and effective treatment modality is systemic therapy. Recent rapid advances in medical therapy, with the discovery of new therapeutic targets and drugs directed at these targets, have led to the concept of stage IV BC as a chronic disease. With the ever-increasing medical therapy armamentarium, and perhaps with better palliative care, survival of patients with metastatic BC has improved steadily over the past two decades [7, 8]. Moreover, improvements in imaging technology, especially combined positron emission tomography with computed tomography (PET-CT), now enable the detection of minute foci of metastases that previously would have remained undetected. Today's patients with metastatic BC are frequently asymptomatic and systemically well controlled. They often have small primary breast cancers rather than locally advanced cancers. Consequently, the question of management of the primary tumor in women with de novo stage IV BC has attracted significant interest, particularly as loss of control at the primary site can have a profound effect on the quality of life. Retrospective data published over the past decade suggest that primary tumor resection and possibly radiotherapy (RT) may improve survival when used in conjunction with effective systemic therapy. These data have led to some new enthusiasm for the resection of asymptomatic primary tumors, in contrast to the classical, dogmatic approach of reserving resection only for palliation of symptomatic primary tumors.

29.1 Arguments Against Primary Surgery in Stage IV Breast Cancer

There are several, historical arguments against performing primary surgery in metastatic BC. Traditional teaching tells us that surgery may not provide any survival advantage, but may be associated with postoperative complications; by not performing surgery, we can avoid potential complications. How surgical procedures can carry inherent risk is well known. There is the possibility of hematoma, infection, and poor healing of the surgical site, particularly if combined with axillary surgery or postmastectomy reconstruction. There is a 16% risk of lymphedema for patients undergoing axillary dissection [9], which is roughly doubled by the addition of radiation following axillary dissection [10]. In the metastatic setting, this may only add to a patient's distress in the last few months or years of life. Moreover, complications from surgery may delay systemic treatment, which is of paramount importance in patients with metastatic disease. This delay in systemic therapy may adversely affect the control of distant disease in some patients. If mastectomy is performed, questions about breast reconstruction arise, which increases the risk of surgical complications and may further delay adjuvant

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therapy [11]; other data suggest that delays in systemic therapy can negatively affect survival. Lohrisch et al. [12] found a decrease in survival when chemotherapy was started more than 12 weeks after surgery in patients with early-stage BC, and this is likely to be even more true of metastatic BC. Finally, women with metastatic disease who are offered primary site surgery are inquiring about (and anecdotally receiving) contralateral prophylactic mastectomy, a clearly inappropriate intervention for this patient population. However, the fact that patients consider it points to the need for definitive, unbiased information to guide treatment plans for a patient population that is highly motivated to pursue any and all options with a possibility of benefit. Apart from the risks and quality-of-life hazards of locoregional therapy for BC, it is also important to remember that this population of patients is often stretched to the limit in terms of out-of-pocket expenses for imaging and therapy, even when insurance plans are generous. Locoregional therapy adds considerably to this burden, which must be considered when arriving at a management plan for this vulnerable population of patients.

Another argument against primary surgery in stage IV BC is that the intact primary breast cancer is easily accessible and provides measurable disease that can be used to gauge the response to systemic treatment; removing this makes the clinical assessment of response to therapy more difficult. Patients with stage IV BC may represent an anesthetic challenge because of debilitation, as well as a surgical challenge because of locally advanced cancer with bulky lymph node involvement; the likelihood of adverse outcomes may be increased. Additionally, in a rodent cancer model, the primary tumor has been shown to inhibit its remote metastases. Following excision of the tumor, neovascularization and growth of the metastases occurred; it is feared that perhaps this could also happen in patients after primary surgery in metastatic BC [13].

29.2 Arguments in Favor of Primary Surgery in Stage IV Breast Cancer

The validity of these arguments against primary surgery in metastatic BC has been apparently challenged. First of all, there have been several studies indicating improvement in survival of women with metastatic BC over the past three decades [8, 14, 15]. A single-institution review of women diagnosed with metastatic disease treated from 1974 to 1979 showed a median survival of 15 months compared with those treated from 1995 to 2000 who had a median survival of 58 months [3]. Similarly, Andre et al. [8] reported temporal trends in improvement of survival for patients with metastatic disease based on treatment period, comparing the intervals 1994–2000 and 1987–1993. Although some of these improvements are undoubted because of better systemic therapy, lead time bias related to more sensitive imaging and therefore earlier diagnosis of metastatic disease in later periods are also likely contributing factors, given the retrospective nature of these studies. More recently, Dawood et al. [15] examined the outcomes between patients with de novo stage IV breast cancer and those who experienced metachronous distant relapse. From a large cohort of patients examined from a single institution, they found the median survival for patients who presented with de novo stage IV breast cancer was 12 months longer than in women with relapsed breast cancer. This difference was statistically significant in both univariate and multivariate analyses. The authors also noted that disease-free interval was also associated with outcomes. Specifically, those patients whose disease relapsed with shorter disease-free interval had worse outcomes when compared with those patients who presented with de novo stage IV disease. The reasons for this difference in outcomes maybe partially related to the fact that women with de novo stage IV disease are treatment naïve and therefore may respond better to systemic therapy, whereas those with metastatic relapse have demonstrated therapeutic resistance of their tumors in the adjuvant setting. There may also be biological differences dictated by the presence of the primary tumor in de novo stage IV disease, as suggested by Retzky et al. [16], vs. reactivation of dormant, resistant clones in metachronous metastases; however, present knowledge regarding interactions between the primary tumor and metastatic sites in humans, and any influence these may have on the course of disease, is limited.

The second argument in favor of primary surgery in metastatic BC comes from evidences accumulated over the past 15 years in non-breast cancer treatment, suggesting that a reduction in tumor burden at the primary site may add to the efficacy of systemic therapy and aid survival. These include a randomized trial of patients with de novo stage IV renal cell carcinoma, which demonstrated a modest but significant survival advantage for the nephrectomy group [17, 18]. An improved survival with resection of primary disease with or without resection of distant disease has also been observed in advanced stages of ovarian cancer [19], in which tumor debulking in the abdominal cavity has become a standard component of overall treatment strategy, despite the lack of a randomized trial testing this approach. Thus, based on retrospective data, these cancers are frequently managed with tumor debulking before chemotherapy [20, 21], drawing on the theory that a smaller tumor burden increases the efficacy of chemotherapy [22]. Again, in colorectal and gastric cancer and in melanoma and sarcoma, resection of the primary tumor, tumor debulking, tumor burden reduction, metastasectomy, re-metastasectomy, multiple metastasectomies, and combined surgery for primary and secondary tumor are widely accepted and routinely performed in clinical practice, being accepted their role in improving survival and quality of life [23–27].

Another theoretical benefit from resection of the primary tumor in patients with overt metastases can be supported along several lines of investigation about the different possible models of progression and metastatic dissemination in BC, ranging from the potential role of BC as a source of tumor stem cells with enhanced metastatic potential [28, 29] to the possibility that tumor-induced immunosuppression is facilitated by the intact primary tumor [30, 31]. It is well known that the progression and metastatic dissemination in breast cancer are a highly selective process that depends on specialized properties of tumor cells (genetically predetermined) and multiple interactions of metastatic cells (seed) with homeostatic mechanism (soil) that tumor cells can exploit. The ongoing seeding from both the primary tumor and distant sites could be an important mechanism of continued tumor growth and metastases. Under this self-seeding theory, tumor cells have the property to escape from the primary tumor and seed distant site but also may metastasize back to the site of the primary tumor [28]. Decreasing the tumor burden could also increase the efficacy of medical treatment by reducing the chances of a resistant clone appearing, and a certain degree of immunomodulation may be achieved by eliminating the immunosuppression associated with the presence of the primary tumor [31]. The newer concepts of the metastatic progression in BC seem to support the concept that the resection of the primary tumor in this scenario would have clinical relevance. In fact, the removal of the primary tumor could theoretically reduce either self-seeding, tumor cell dissemination, or the population of native cancer stem cells, finally making more effective the systemic therapy. Conversely, there has been a concern, based on laboratory data, that primary tumor resection may accelerate the growth of metastatic lesions, but this has not been demonstrated in humans. Although these laboratory data suggest a biological basis for improved survival with resection of the primary tumor in the setting of metastatic disease, these specific models have not been validated in humans. In conclusion, it would be naïve to believe that surgery will benefit all women with metastatic breast cancer [32], but in theory no clinical or biological reason exists to exclude a priori the surgery in all patients with stage IV breast cancer.

Finally, there are important quality-of-life (QOL) hazards that relate to the primary tumor, regardless of whether primary site local treatment is used. If the intact primary tumor progresses, the QOL effect of uncontrolled chest wall disease can be disastrous for a minority of women [33, 34]. Actually, for most women with intact primary tumors and distant disease that is responsive to systemic therapy, the primary tumor tends to remain controlled and asymptomatic with medical therapy [35]. So, if all women were subjected to primary site local treatment, most of them would experience the QOL risks of surgery and potentially RT, including those who would not have developed uncontrolled local disease during the remainder of their lives. Therefore, the analysis of the QOL effect has to be very thoughtful, weighing the possibility of uncontrolled local disease in a minority of women against the potentially unnecessary costs of primary site local treatment in all women.

29.3 Retrospective Studies and Meta-analysis on Primary Tumor Resection

Based on these considerations and mainly following the publication of a randomized trial demonstrating the value of primary tumor resection in stage IV renal cell carcinoma, a number of retrospective studies were performed to examine the effect of surgical resection of the primary tumor on survival in the setting of metastatic BC [1, 2, 4, 33, 36–46]. These studies have come from single institutions and large data bases from the United States, Europe, and Asia. The type of local therapy has largely been a surgery alone, although a few authors have been able to evaluate surgery plus RT [47–49]. The survival outcomes in these retrospective analyses have been the subject of several reviews and meta-analyses [50–54].

A large meta-analysis by Petrelli and Barni published in 2012 [51] included 15 retrospective case series and found that surgery of the primary tumor was independently associated with longer survival, with a hazard ratio (HR) of 0.69 ($P < 0.00001$) (Fig. 29.1). On overall, surgery reduced the risk of death by 30%, especially when it was associated with systemic therapy and RT in a multimodality strategy. The survival benefit was independent of age, tumor burden, type of surgery, margin status, site of metastases, hormone receptor status, and HER2 status; the use of systemic therapy and RT was significantly associated with survival.

A similar literature has developed on the use of primary RT for the primary site, showing a similar magnitude of survival benefit. The RT studies have come mainly from single institutions in France and Canada. The first and largest was reported by Le Scodan et al. [47]. These investigators identified 581 patients with de novo stage IV BC treated between 1984 and 2004, 320 of whom received RT, with 41 women receiving both surgery and RT and 30 receiving only surgery. Nodal fields were included for most patients, and most of those receiving RT were given a boost dose to the tumor site. The overall survival rate was 43% in the group receiving locoregional therapy vs. 27% in those who did not, for an adjusted HR = 0.7 (95% CI: 0.58–0.85). A second French study of 236 patients described similar differences in outcomes with the use of primary RT to the primary site, but adjusted estimates of overall survival showed no significant advantage for the primary site local treatment group [48]. The value of postoperative RT has been difficult to assess in these retrospective studies, as large databases such as the

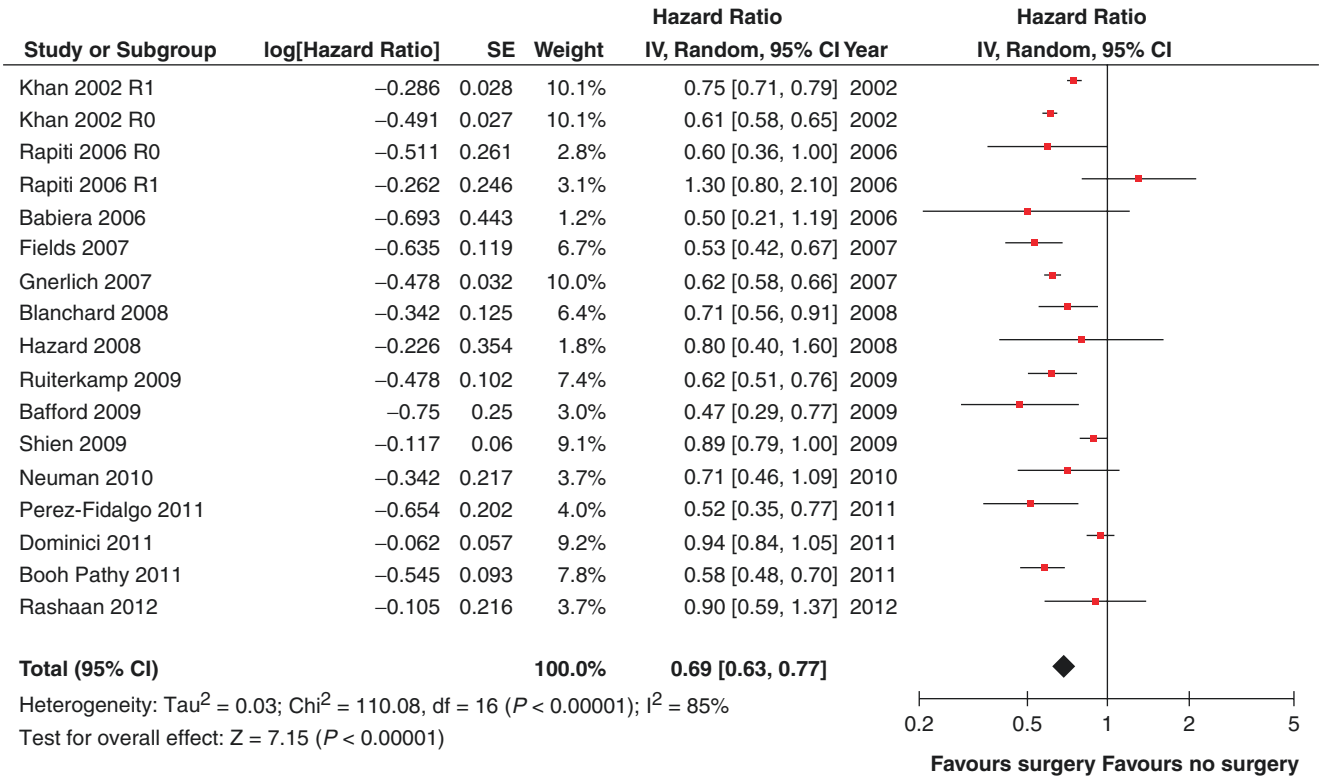

Fig. 29.1 Meta-analysis on 15 retrospective case series, from Petrelli et al. [51]

National Cancer Database (NCDB) and the Surveillance, Epidemiology, and End Results (SEER) did not distinguish between RT to the primary and metastatic sites. The data that are available do not allow clear conclusions and do not show a significant advantage to the combination of surgery and RT to the primary site.

A larger systematic review and meta-analysis were published in 2016 by Headon et al. [55]. The analysis included 16 studies and compared 15,368 stage IV BC patients submitted to surgery of the intact primary tumor to 14,313 not operated patients. In this meta-analysis, a pooled hazard ratio of 0.63 (95% confidence interval, 0.58–0.7; $P < 0.0001$) was revealed, equating to a 37% reduction in risk of mortality in patients that underwent surgical resection of the primary tumor (Fig. 29.2). The conclusions, consistent to what previously reported, are that surgery of the primary tumor in stage IV BC apparently offers a survival benefit in metastatic patients.

29.4 SEER (Surveillance, Epidemiology, and End Results) Data on Primary Tumor Resection

In 2016, four different analyses on primary site local therapy in stage IV BC, based on SEER data, were published. Eng et al. [56] retrieved the records of 25,323 women diagnosed with primary stage IV BC in the SEER 18 registries database from 1990 to 2012. For each case, information on age at diagnosis, tumor size, nodal status, estrogen receptor status, progesterone receptor status, ethnicity, cause of death, and date of death were extracted. The Cox proportional hazard model was used to estimate the unadjusted and adjusted hazard ratio (HR) of death due to stage IV BC, according to age group. Among 25,323 women with stage IV BC, 2542 (10.0%) were diagnosed at age 40 or below, 5562 (22.0%) were diagnosed between ages 41 and 50, and 17,219 (68.0%) were diagnosed between ages 51 and 70. After a mean follow-up of 2.2 years, 16,387 (64.7%) women died of breast cancer (median survival 2.3 years). The 10-year actuarial breast cancer-specific survival rate was 15.7% for women ages 40 and below, 14.9% for women ages 41–50, and 11.7% for women ages 51 to 70 ($p < 0.0001$). In an adjusted analysis, the risk of death from BC at 10 years was significantly lower for women ages 40 and below (HR 0.78; 95% CI, 0.74–0.82; $p < 0.0001$) and for women ages 41–50 (HR 0.82; 95% CI, 0.79–0.85; $p < 0.0001$), compared to women ages 51–70. The authors concluded that approximately 13% of women with primary stage IV breast cancer survive 10 years after diagnosis. Women diagnosed with stage IV BC before age 50 have better survival at 10 years compared to older women.

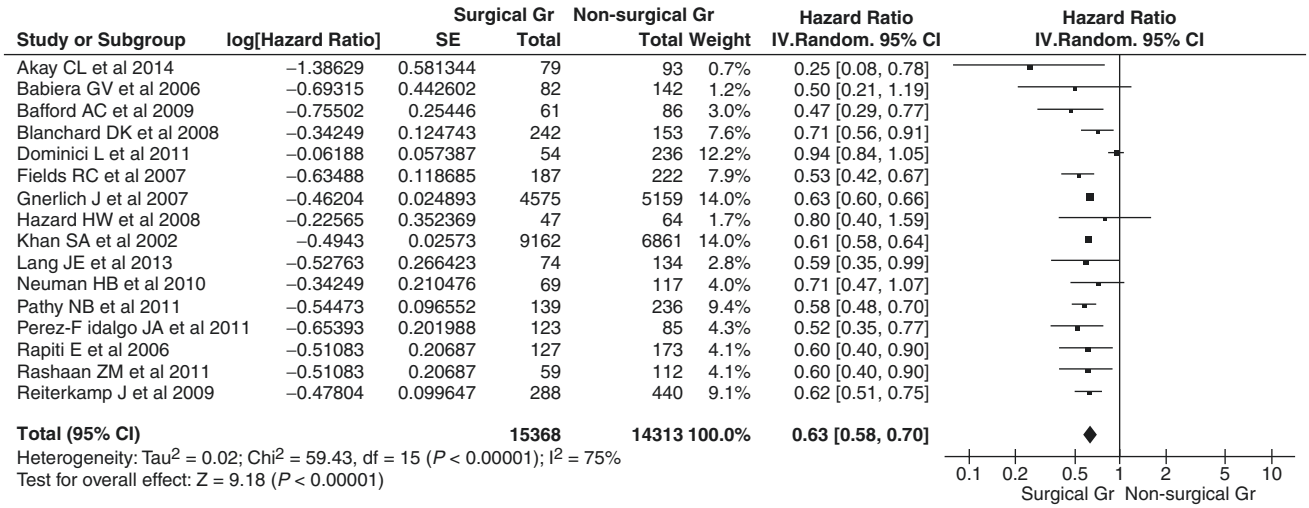

Fig. 29.2 Meta-analysis on 16 retrospective case series, from Headon et al. [55]

Similarly, Warschkow et al. [57] assessed the effect of primary tumor surgery on overall and cancer-specific mortality using risk-adjusted Cox proportional hazard regression modeling and stratified propensity score matching in metastatic BC patients identified in the SEER registry between 1998 and 2009. Overall, 16,247 women with metastatic BC were included. Of those, 7600 women underwent primary tumor surgery although 8647 did not have any surgery at all. Primary tumor surgery decreased from 62.0% in 1998 to 39.1% in 2009 ($P < 0.001$). Primary tumor surgery was associated with decreased overall mortality (hazard ratio (HR) = 0.53, 95% CI 0.50–0.55, $P < 0.001$) and cancer-specific mortality (HR = 0.51, 95% CI 0.48–0.54, $P < 0.001$) in the propensity score-matched model. The benefit of primary tumor surgery increased from 1998 to 2009 for overall mortality (1998, HR = 0.72, 95% CI 0.59–0.89; 2009, HR = 0.42, 95% CI 0.35–0.50) and cancer-specific mortality (1998, HR = 0.72, 95% CI 0.58–0.89; 2009, HR = 0.40, 95% CI 0.33–0.48). In conclusion, the study provided evidence of a favorable impact of primary tumor surgery on mortality in metastatic BC patients. Most importantly, the benefit of primary tumor surgery increased over time from 1998 to 2009.

In order to evaluate the patterns of receipt of initial breast surgery for female patients with stage IV BC in the United States, with particular attention to women who survived at least 10 years, Thomas et al. [58] analyzed a retrospective cohort of patients using data from the SEER program. Female patients diagnosed as having stage IV BC between 1988 and 2011 and who did not receive RT as part of the first course of treatment were included (n = 21,372). Kaplan-Meier estimates of median survival and descriptive statistics were used to compare patient and tumor characteristics by receipt of breast surgery at diagnosis. A Royston-Parmar survival model and logistic regression analysis assessed demographic and clinical factors associated with survival and prolonged survival (of at least 10 years). Mail outcome of the analysis was differences in survival, particularly survival of at least 10 years, by receipt of initial surgery to the primary tumor. Among the 21,372 patients, the median survival increased from 20 months (1988–1991) to 26 months (2007–2011). During this time, the rate of surgery declined (odds ratio [OR], 0.16; 95% CI, 0.12–0.21). Even so, receipt of surgery was associated with improved survival in multivariate analysis, which controlled for patient and clinical characteristics, along with time period (hazard ratio, 0.60; 95% CI, 0.57–0.63). For women diagnosed as having cancer before 2002 (n = 7504), survival of at least 10 years was seen in 9.6% (n = 353) and 2.9% (n = 107) of those who did and did not receive surgery, respectively (OR, 3.61; 95% CI, 2.89–4.50). In multivariate analysis, survival of at least 10 years was associated with receipt of surgery (odds ratio, 2.80; 95% CI, 2.08–3.77), hormone receptor-positive disease (OR, 1.76; 95% CI, 1.25–2.48), older age (OR, 0.41; 95% CI, 0.32–0.54), larger tumor size (OR, 0.37; 95% CI, 0.27–0.51), marital status of being separated at the time of diagnosis (OR, 0.67; 95% CI, 0.51–0.88), and more recent year of diagnosis (OR, 1.43; 95% CI, 1.02–1.99). In conclusion, survival in stage IV BC was improved and was increasingly of prolonged duration, particularly for some women undergoing initial breast surgery.

Finally, Tan et al. [59] used the SEER database to explore the impact of surgery on the survival of patients with stage IV BC and included 10,441 eligible stage IV BC patients from 2004 to 2008. They were divided into four groups as follows: R0 group (patients who underwent primary site and distant metastatic site resection), primary site resection group, metastases resection group, and no resection group. The four groups achieved a median survival time of 51, 43, 31, and 21 months, respectively, $P < 0.001$. The Cox proportional hazard model showed that the R0 group, primary

resection group, and metastases resection group had a good survival benefit, with hazard ratios of 0.558 (95% CI, 0.471–0.661), 0.566 (95% CI, 0.557–0.625), and 0.782 (95% CI, 0.693–0.883), respectively. In the hormone receptor (HR)-positive population, the R0 group (median survival time = 66 m, 5-year OS = 54.1%) gained an additional survival benefit compared with the primary resection group (median survival time = 52 m; 5-year OS = 44.9%; $P < 0.001$). The metastases resection group (median survival time = 38 m; 5-year OS = 31.7%) survived longer than the no resection group (median survival time = 28 m; 5-year OS = 22.0%; $P < 0.001$). In the HR-negative population, the R0 group and primary resection group had a similar survival ($P = 0.691$), and the metastases resection group had a similar outcome to that of the no resection group ($P = 0.526$) (Fig. 29.3). In conclusion, patients who underwent surgery for stage IV BC showed better overall survival than the no resection group, especially when cytoreductive surgery is performed in HR+ stage IV BC patients.

29.5 Potential Biases of Retrospective Studies and Meta-analysis

Many potential biases could have affect and spoiled the enthusiastic results of all the retrospective studies, including meta-analysis. First of all, the timing of surgery on the primary tumor in relation to the diagnosis of metastases, and the use of systemic therapy, has not always been specified in the published retrospective literature, although several authors have attempted to address it [41, 46, 60, 61], with varying conclusions. This is a source of bias because women who are

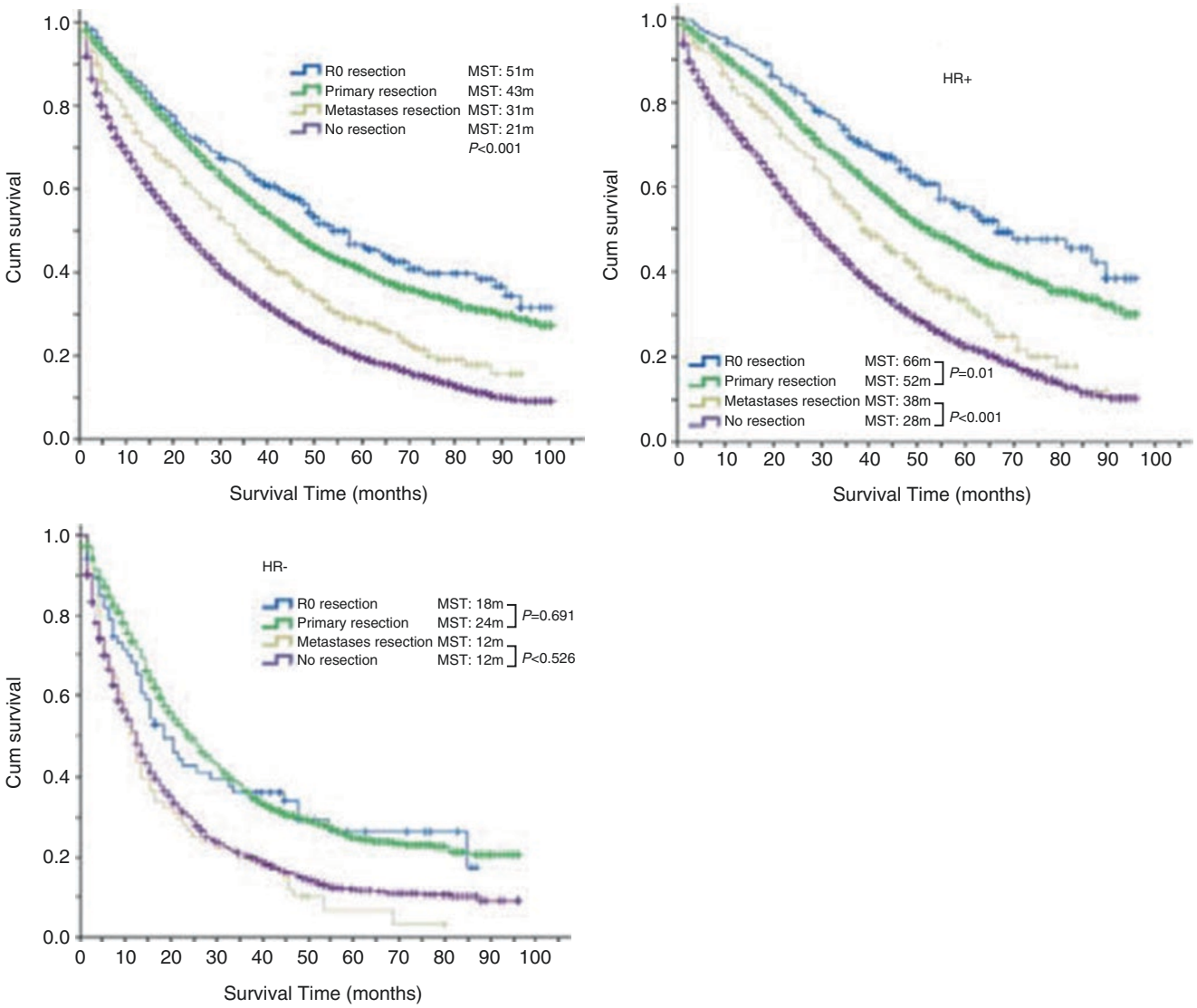

Fig. 29.3 (**a**) Overall survival curves in four groups, (**b**) Kaplan-Meier survival curves of the four groups in the HR+ population (**c**) and in HR-population, from Tan et al. [59]

diagnosed with metastases only after they have undergone surgery for the primary tumor most likely have asymptomatic (and therefore lower-volume) metastases. In contrast, those who present with symptomatic metastases, discovered before surgery, are likely to have a higher burden of distant disease. In reviewing the available literature, we see that there is a substantial fraction of women with T1–T2 tumors, raising the possibility that these women were operated on with the assumption of nonmetastatic disease, were diagnosed with metastases postoperatively, and were then classified as having stage IV disease during the abstraction process by local tumor registrars. Other sources of bias exist in the retrospective data although all studies have attempted to control for these using multivariate regression. In fact, single-institution studies are limited by institutional biases and small numbers of well-selected patients, often treated during a long period of time and with different, and differently effective, medical therapies. In addition, some studies were multi-adjusted with exclusion of patients with short survival, delayed metastatic disease, and more advanced tumors.

Although the meta-analysis seems to definitively confirm the positive impact of surgery of the primary tumor on survival in stage IV patients, it well known that the assumption that a meta-analysis uniformly represents the final and accurate viewpoint of an area of research is not warranted. A meta-analysis combines similar trials in order to obtain a larger number of patients to improve the evaluation of whether statistically reliable differences exist between comparison groups. Meta-analyses are by no means perfect. The conclusions made by the authors of a meta-analysis are subject to the same potential for bias as the smallest of clinical studies included in it. On some occasions, a large clinical trial has subsequently been performed evaluating the same clinical question with an outcome quite different from the initial meta-analysis, and discrepancies between meta-analyses and subsequent large randomized clinical trials are documented in literature [62]. In all the retrospective studies on stage IV BC included in the recent meta-analysis, women who received surgery tended to be younger [33, 36, 37, 39, 40, 44, 46], tended to have smaller tumors [2, 4, 36, 38–40, 42], tended to have fewer comorbidities and better performance status [2, 44, 45], tended to have a lower burden of metastatic disease [2, 36–38, 41, 42, 44–46], were less likely to have visceral metastases [2, 36, 38, 43, 45], and were likely to have better access to care [38, 40]. Meta-analysis simply reflects the biases of the retrospective studies considered in it.

Finally, studies based on large population-based data sets such as administrative claim data and tumor registry data (SEER data on metastatic BC) have become increasingly common in surgical oncology research. These data sets can be acquired relatively easily, and they offer larger sample sizes and improved generalizability compared with institutional data. There are, however, significant limitations that must be considered in the analysis and interpretation of such data. Invalid conclusions can result when insufficient attention is paid to issues such as data quality and depth, potential sources of bias, missing data, type I error, and the assessment of statistical significance [62]. In fact, the population database studies on metastatic BC incorporated large cohorts of very heterogeneous patients treated during a long period of time and provided the most generalizable conclusions, but they were limited by the clinical variables recorded (HER-2/neu status, indications for the procedure, the specific procedure performed, time of surgery, surgical margin status, systemic therapy, and local RT). So, registry data should be interpreted with caution and good understanding of its limitations.

In conclusion, these consistent biases related to retrospective data and large population-based registries on metastatic BC, including meta-analysis, raise real questions as to whether the better survival of women undergoing primary site local treatment is a cause-and-effect relationship or simply means that physicians are good at picking out patients who are likely to survive longer and definitively selecting patients with the best prognosis at all. Therefore, any conclusions reached from these series should be considered exploratory, and physicians should therefore exercise appropriate caution in adopting these data to their therapeutic strategies, as the survival value of primary site local treatment clearly remains to be proven.

29.6 The Clinical Trials

The initial wave of retrospective data suggesting a survival advantage to primary tumor resection, the recognition of consistent biases observed in the published retrospective analyses, and the potential for harm from surgical and RT interventions led to the launching of seven randomized trials in different countries (Table 29.1). Of these, two trials are ongoing (Japan and Austria), two have been completed (India and Turkey), one has just completed enrollment (the Unites States and Canada), one has terminated for lack of enrollment (the Netherlands), and another was withdrawn before enrollment (Thailand). The two completed trials were presented at the San Antonio Breast Cancer Symposium (SABCS) in 2013 [63, 64, 66], and one (India) was recently published [64]. These presentations can be viewed at http://www.sabcs.org/PastSymposia/Index.asp#SABCS2013. Their major features are compared in the Table 29.2. In addition to the main question of whether locoregional therapy is beneficial to women with stage IV BC, these trials address the many ancillary questions regarding the selection of patients who may benefit from resection of the primary tumor, the value of surgery alone vs. surgery plus RT, the type of surgery (mastectomy vs. tumorectomy), and the optimal timing of surgery.

Table 29.1 Completed and ongoing randomized trials testing locoregional therapy with systemic therapy vs. systemic therapy alone

Country	Accrual period	Sample size	Initial therapy	Radiotherapy	Status
India [63, 64] NCT00193778	2005–2012	350	Chemotherapy	If indicated	Closed, published
Japan [65] JCOG 1017	2011–2016	410	Systemic therapy	Not addressed	Enrolling
US and Canada EA2108 NCT01242800	2011–2015	368	Systemic therapy	Per standards for stages I–III	Closed July 2015
Turkey [66, 67] NCT00557986	2008–2012	271	Surgery	For breast conservation	Closed, F-up immature
Netherlands [68] SUMBIT trial NCT01392586	2011–2016	516	Surgery	For positive margins or palliation	Closed. Lack of enrollment
Austria POSYTIVE trial NCT01015625	2010–2019	254	Surgery	Per standards for stages I–III	Enrolling

Table 29.2 Comparison of randomized Indian and Turkish trials presented at SABCS 2013

	Tata memorial (2005–2012)	Turkey MF 07-01 (2008–2012)
Randomization time point	After systemic therapy	At diagnosis
Number randomized	350	271
Primary end point	Overall survival	Overall survival
Stratification	Number and type of metastases and hormone receptor	None described
Preplanned subset analysis	Age, ER and HER2 status, number, and type of metastases	None described
Tumor size	Not described	More T2 and T4 tumors in systemic therapy arm
Receptor status	Balanced	More triple-negative tumors in systemic therapy arm
Metastatic Burden	Balanced >3 lesions	Single organ site more frequent in surgical arm
Bone-only metastases	Balanced	Fewer in systemic therapy arm
Hazard ratio for survival	1.04 (95% CI: 0.8–1.3)	0.76 (95% CI: 0.49–1.16)
Hazard ratio for local control	0.16 (95% CI: 0.10–0.26)	Too few events

29.7 Trials Requiring Randomization to Local Therapy After Systemic Therapy

The rationale for initial systemic therapy is based on the notion that PSLT can provide survival value only if disease at distant sites is responsive to systemic therapy. There are four trials that have been initiated that adopt this approach.

The first trial to open was in India, at the Tata Memorial Cancer Institute in Mumbai, in 2005 (NCT00193778), and was published in 2015 [64]. In this open-label, randomized controlled trial, previously untreated patients (≤65 years of age with an estimated remaining life expectancy of at least 1 year) presenting with de novo metastatic BC were recruited. Patients were randomly assigned (1:1) to receive locoregional treatment directed at their primary breast tumor and axillary lymph nodes or no locoregional treatment. Randomization was stratified by site of distant metastases (bone, viscera, or both), number of metastases (more than 3 vs. <3), and hormone receptor status of the tumor. Use of induction endocrine therapy occurred in 4% of patients in each arm. Patients with resectable primary tumor in the breast that could be treated with endocrine therapy were randomly assigned upfront, whereas those with an unresectable primary tumor were planned for chemotherapy before randomization. Of the patients who had chemotherapy before randomization, they randomly assigned patients who had an objective tumor response after six to eight cycles of chemotherapy. The primary end point was an overall survival analyzed by intention to treat. The trial was powered to detect a 6-month improvement in 2-year survival (from 18 to 24 months). Between February 2005 and January 2013, of the 716 women presenting with de novo metastatic BC, 350 patients were randomly assigned: 173 to locoregional treatment and 177 to no locoregional treatment. At data cutoff of November 2013, median follow-up was 23 months (IQR 12.2–38.7) with 235 deaths (locoregional treatment $n = 118$, no locoregional treatment $n = 117$). Median overall survival was 19.2 months (95% CI, 15.98–22.46) in the locoregional treatment group and 20.5 months (16.96–23.98) in the no locoregional treatment group (HR 1.04; 95% CI, 0.81–1.34; $p = 0.79$), and the corresponding 2-year overall survival was 41.9% (95% CI, 33.9–49.7) in the locoregional treatment group and 43.0% (35.2–50.8) in the no locoregional treatment group (Fig. 29.4). The only

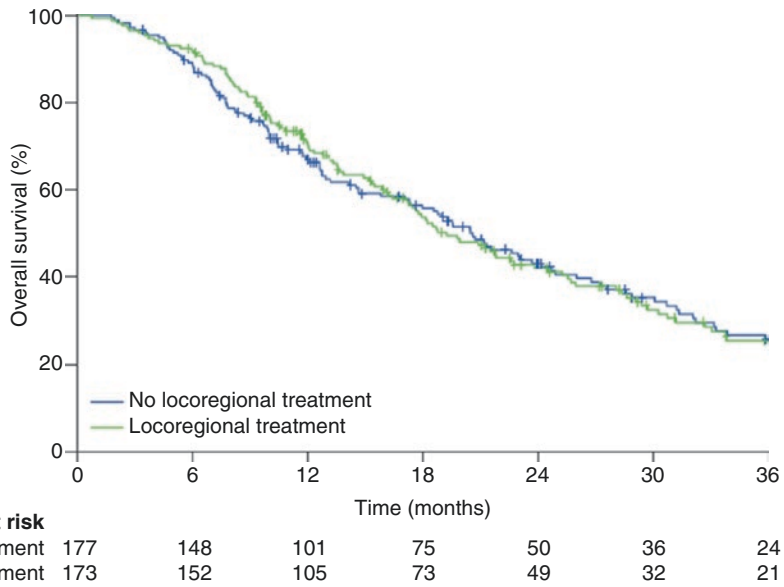

Fig. 29.4 Kaplan-Meier plot of overall survival (From: Badwe et al. [64])

adverse event noted was wound infection related to surgery in one patient in the locoregional treatment group. The authors concluded that there is no evidence to suggest that locoregional treatment of the primary tumor affects overall survival in patients with metastatic BC at initial presentation who have responded to frontline chemotherapy and this procedure should not be part of routine practice. Planned subset analyses examined survival outcomes for premenopausal vs. postmenopausal women, those with bone-only metastases vs. those with bone plus visceral metastases, more than 3 vs. 1–3 metastatic lesions, and hormone receptor or HER2 subsets, with no significant differences noted. However, where the odds ratio deviated from unity, it favored the systemic therapy only arm (1.4 for bone-only disease and 1.6 for 3 or fewer metastases) (Fig. 29.5). It is noteworthy that the usual approach to systemic therapy for the population in this trial (per Indian standards) was that following induction, therapy was not continuous. Therapy was suspended following good response or stable disease and was resumed on progression. This may explain the difference in 3-year survival seen in this trial and the registry trial in the United States [69] (TBCRC 0013, discussed later). As expected of course, the local progression-free survival rate was significantly better in the surgical group (80% at 5 years compared with 20% in the nonsurgical group, $P < 0.001$).

Currently, two trials with similar design are ongoing. In Japan, JCOG 1017 [65] seeks to enroll 410 patients with newly diagnosed metastatic BC to compare the efficacy of primary tumor resection plus systemic therapy vs. systemic therapy alone. After 3 months of systemic therapy, women who show no disease progression are randomized to undergo surgery or to continue systemic therapy; RT is not required. The primary outcome is overall survival. Secondary outcomes are local recurrence rate, local control rate, and effect on distant metastasis after resection of the primary site. More than 350 patients have been enrolled to date.

In the United States and Canada, the Eastern Cooperative Oncology Group EA2108 (NCT01242800) recruited 383 patients with metastatic BC (revised downward from 880 patients because of slow enrollment), and the trial was closed on July 30, 2015. All patients receive induction systemic therapy at the discretion of the treating physician, consisting of endocrine, cytotoxic, or biologic regimens appropriate to the patient's age and tumor type. Patients without progression of disease after 16–32 weeks of treatment were randomized to either locoregional therapy including surgery and RT (to mirror standards of treatment for patients with nonmetastatic disease) or the continuation of systemic therapy. Randomization was stratified by the type of induction systemic therapy (endocrine therapy, chemotherapy, or chemotherapy with anti-HER2 agents), as that would also reflect the biological subtype of the tumor. It was estimated that 80% of registered patients would respond or demonstrate stable disease and would proceed to randomization, and a crossover of 15% was built into the design (anticipating that some patients would not accept the locoregional therapy arm that they are assigned to). Therapy for the primary site was allowed for palliation, later in the course of disease, for women who were randomized to the systemic therapy–alone arm. The primary outcome is survival; the trial is powered to detect an overall survival difference of 19% at 3 years (from 30 to 49%). The secondary outcomes are local progression-free survival and quality of life, and biological samples are being banked for correlative studies. The fraction of patients dropping out for disease progression during induction systemic therapy, and the fraction of crossing over, is within the expected range. Results are expected by 2017.

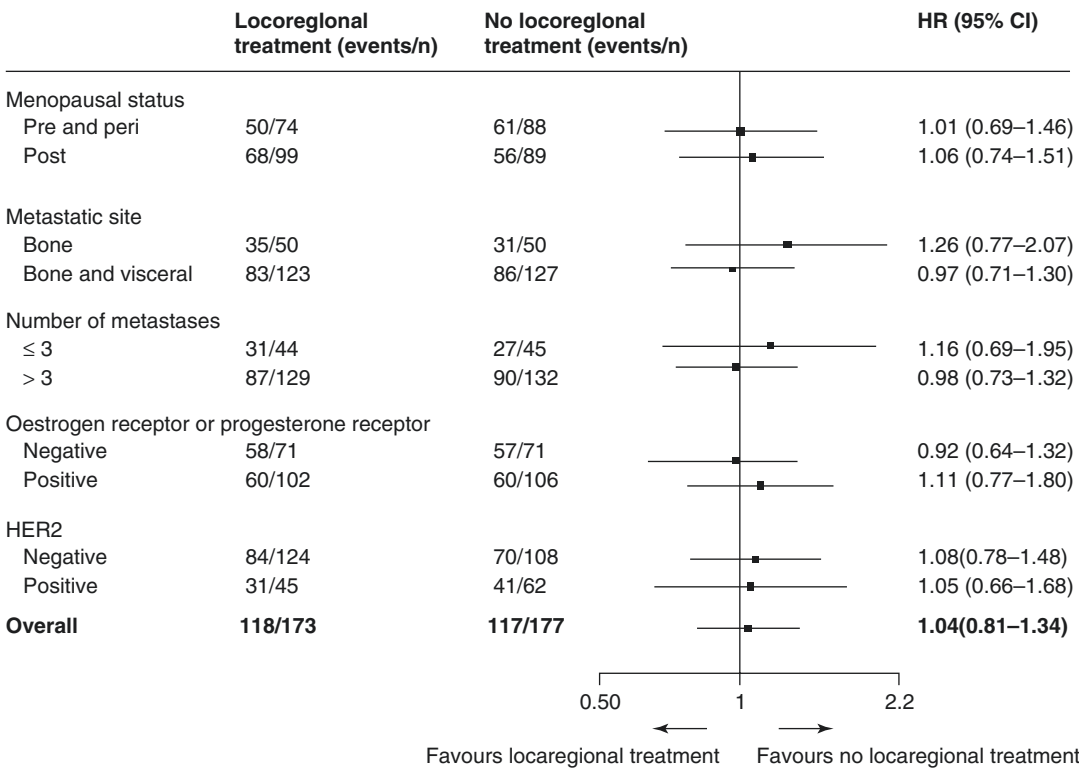

Fig. 29.5 Forest plot of overall survival subgroup analysis unadjusted hazard ratios. (From: Badwe et al. [64])

29.8 Trials Requiring Randomization to Locoregional Therapy Before Systemic Therapy

Some have argued that randomization to, and delivery of, locoregional therapy before systemic therapy is a purer test of the potential benefit of primary site local treatment in the metastatic setting, as it avoids selection of only those patients who respond to systemic therapy. Although this approach avoids the "bias" of including only responders to systemic therapy, it also means that primary site local treatment is provided to some patients whose tumors are unresponsive to systemic therapy at distant sites and therefore will die early owing to their distant disease. The first trial with this design was opened by the Turkish Federation of Breast Diseases (NCT00557986) and has completed enrollment, with early data reported at SABCS 2013 [67]. The design included randomization to surgery before systemic therapy or systemic therapy alone with RT to the primary site in cases of breast conservation. The trial was powered to detect an improvement in the 3-year survival rate from 17 to 35%. A total of 271 evaluable women were recruited. The primary outcome was overall survival, with secondary outcomes related to progression-free survival, quality-of-life measures, and morbidity related to locoregional therapy. The trial was reported at SABCS 2013 with a median follow-up of 18 months and 31% of the population having died [66]. No significant survival advantage was observed at the time of reporting, with a median survival of 42 vs. 46 months, favoring surgery (HR = 0.76; 95% CI, 0.49–1.16; $P = 0.2$). Multiple unplanned subset analyses revealed only the possibility of an advantage with surgery for women with solitary bony metastases (33 women in the primary site local treatment arm and 20 in the control arm; HR = 0.23, $P = 0.02$), but these were not biopsy proven at entry. There were insufficient local recurrences for analysis.

The SUMBIT trial in the Netherlands (NCT01392586) was similarly structured but closed after enrollment of ten patients [68]. The POSYTIVE trial in Austria (NCT01015625) has a roughly similar design and aims to recruit 254 patients with synchronous metastatic breast cancer and randomly assign them to receive either PSLT (lumpectomy or mastectomy + axillary surgery/± RT) vs. nothing. This has recently been revised to allow systemic therapy before randomization. Primary outcomes are median survival. Secondary outcomes are time to distant progression and time to local progression.

29.9 Prospective Registry Study TBCRC 0313

A prospective study entitled "A Prospective Analysis of Surgery in Patients Presenting with Stage IV Breast Cancer" is a multi-institutional data registry [69]. The objective is to

characterize patients with stage IV BC while documenting clinical management outcomes. The planned total enrollment is 100 patients. Its primary aims are to document response to first-line therapy, frequency of surgical referral, and proportion of patients undergoing surgery, determine the incidence of uncontrolled local disease and frequency of surgical palliation, and correlate molecular characteristics of primary tumor with conventional prognostic factors. A number of correlative molecular studies of circulating tumor cells and analyses of primary and metastatic tumor samples are planned. The analysis of registered patients with stage IV disease follows two tracks: one for those with an intact primary and one for those with the primary tumor resected and metastases discovered within 3 months of surgery. Local and distant disease will be carefully monitored in women with responsive and nonresponsive disease, and the use of research biopsies during therapy will provide samples to generate biological hypotheses related to interactions between responding and nonresponding primary and metastatic sites, the frequency and effect of uncontrolled chest wall disease, quality of life, and other issues. As reported at the San Antonio Breast Cancer Symposium in 2013, the 3-year survival rate of 39 women who were responsive to induction systemic therapy and received surgery was 81%, whereas the survival rate of responders who did not receive surgery was 79%. Although the numbers are small, it is also of interest that among 15 women who were diagnosed with metastases following surgical therapy for the primary tumor, the 3-year survival rate was 87%. These numbers are remarkably different from the survival experience in the Tata Memorial trial (3-year survival rate of 25%) and point to the differences in the biological and therapeutic environment between Indian and US populations.

29.10 Recommendations

Although retrospective data suggest that locoregional therapy for the primary tumor may provide a survival advantage in women with metastatic BC and an intact primary tumor, this is not confirmed by randomized trials. The biases of the retrospective data include the use of surgery in younger women with smaller tumors, single sites of metastasis, and less visceral disease. The publication of data from the Tata Memorial [64] and Turkish Federation [67] trials provides information on patients treated prospectively. These and ongoing trials will allow us to evaluate the role of surgery alone or surgery plus RT and will allow us to reach solid conclusions regarding the role of locoregional therapy, how extensive it should be, and its timing in stage IV breast cancer.

Until additional unbiased data are available, surgery and RT to patients with stage IV with an intact, asymptomatic primary tumor cannot be recommended outside a clinical trial. In particular, there is no basis for recommending surgery to women with distant disease (a) if the distant disease is not well controlled, as survival will likely not be long enough for the primary site to become a problem, or (b) both local and distant sites are well controlled, in which case, the primary site is likely to remain well controlled for the patient's life span. A possible exception to these rules may be a patient who would be rendered as having stage IV NED by resection of the primary tumor, although this too is based on highly selected series. For a patient whose distant disease is controlled but the primary site is progressing, surgery provides a reasonable approach [70].

Locoregional therapy for the primary tumor should be offered to patients only with full disclosure of the lack of evidence of a survival benefit. If a clinical trial is available and the patient is willing to consider it, that is clearly the most rational choice. If primary site local treatment is decided on following the considerations described earlier, the subset of patients that may benefit from more aggressive local therapy ("ideal" patients for primary site local treatment), based on the data from retrospective series and population database studies, includes young patients with good performance status (women diagnosed with stage IV breast cancer before age 50 have better survival at 10 years compared to older women) [56], smaller primary tumor, ER-/PR-positive or HER-2/neu amplified tumor (in which more effective targeted therapies are available), oligometastatic disease (solitary and or low-volume metastatic disease) [59], and possibly with metastatic spread limited to the skeleton [36, 37, 71].

If primary site local treatment is planned, either mastectomy or lumpectomy is appropriate, but of course breast conservation (if feasible) is clearly the least harmful option, and the odds of successful breast conservation can be maximized by the use of effective systemic therapy preoperatively. In case of breast-conserving surgery (BCS), resection margins were strongly considered in the past as important prognostic factor of local recurrence and survival. The original report by Khan et al. found that for BCS or mastectomy patients, the median 3-year survival was 35–36% for patients with clear margins, 26% for those with positive margins, and 17% in nonsurgical patients ($p < 0.0001$) [1]. Two further tumor registry studies [36, 72] and an institutional study from Malaysia [4] also demonstrated that survival was improved in patients with negative margins. These findings may be easily explained by differences between groups, and currently the true significance of the resection margins in BCS has been strongly resized, either in early breast cancer [73] or in metastatic BC. So, although we should always aspire to clear surgical margins when we perform BCS in metastatic BC, this is not mandatory and a re-excision for involvement of the resection margins should be always

avoided. In case of mastectomy, plastic reconstruction is not absolutely contraindicated and must be evaluated case by case, considering the possible immunodepression related to larger reconstructive surgery.

The data on axillary surgery are extremely limited, but if surgery is undertaken, removal only of all gross and symptomatic disease seems prudent. Out of these, routine axillary dissection, axillary nodal samples, and sentinel lymph node biopsy should be categorically avoided, being unnecessary neither for prognostic information nor for local control of the disease.

The evidence supporting the use of postoperative RT is weak [47, 48], at best, and cannot be recommended presently. Primary RT can be considered with the same caveats as surgical resection, particularly if the surgical procedure required would be mastectomy. Although whether RT should follow surgery is unproven, its use may be justified if the risk of early local recurrence and uncontrolled chest wall disease is high.

Conclusions

Stage IV disease is a chronic and incurable disease, and the goals of treatment are only the prolongation of life and the palliation or prevention of symptoms. In stage IV breast cancer patients, the primary and most important and effective treatment modality still remains a systemic therapy. The retrospective data suggest that locoregional therapy for the primary tumor may provide a survival advantage in women with metastatic BC and an intact primary tumor, but this is not confirmed by randomized trials. The publication of data from the Tata Memorial [64] and Turkish Federation [67] trials provides information on patients treated prospectively. These and other ongoing trials will allow us to evaluate the role of surgery alone or surgery plus RT and will allow us to reach solid conclusions regarding the role of locoregional therapy, how extensive it should be, and its timing in stage IV breast cancer. Until additional unbiased data are available, surgery and RT to patients with stage IV with an intact, asymptomatic primary tumor shouldn't be routinely performed or recommended outside a clinical trial. Only for selected patient whose distant disease is controlled by systemic therapy but the primary site is progressing, surgery provides a reasonable approach.

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Breast Cancer in Lymphoma Survivors

30

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30.1 Lymphoma Survivors

Non-Hodgkin lymphoma (NHL) is the most common hematologic malignancy in the world [1]. It is more common in developed countries, with an estimated 70,800 new cases in the United States (US) in 2014. Accounting for 4.3% of all cancers in the US, NHL ranks as the seventh most common cancer among males and the sixth most common cancer among females. NHL consists of more than 40 major subtypes with distinct genetic, morphologic, and clinical features. The incidence of NHL subtypes also varies by age, sex, ethnicity, and geographic region [2, 3]. Hodgkin lymphoma (HL) affects approximately 9050 new patients in the US each year [4], representing approximately 11.2% of all lymphomas. The disease has a bimodal distribution with an increased incidence in young adults as well as in patients 55 years and older [5].

Since the late 1960s, when combination chemotherapy and high-energy radiation therapy were introduced for the treatment of lymphomas, survival of patients has improved dramatically over the time, increasing from 87% in 1975 through 1979 to 97% in 2003 through 2009 [6]. In recent statistics [7], the American Cancer Society and the National Cancer Institute collaborated to estimate the number of current and future cancer survivors using data from the Surveillance, Epidemiology, and End Results (SEER) cancer registries. Incidence and survival were modeled by cancer type, sex, and age group using invasive malignant cases diagnosed from 1975 through 2012 from the nine oldest registries in the population-based SEER program. Among more than 15.5 million Americans with a history of cancer alive on January 1, 2016, there are an estimated 219,570 HL survivors and 686,370 NHL survivors. Although both HL and NHL occur in children and adults, the majority of HL cases (64%) are diagnosed before the age of 50 years, whereas most NHL cases (85%) occur in those aged 50 years and older.

30.1.1 Treatment and Survival for HL and NHL

There are two major types of HL. Classical HL (CHL) is the most common and is characterized by the presence of Reed-Sternberg cells. Nodular lymphocyte-predominant HL (NLPHL), which is characterized by "popcorn cells," comprises only about 5% of cases [8]. NLPHL is a more indolent disease with a generally favorable prognosis [9]. CHL is generally treated with multiagent chemotherapy (88%), sometimes in combination with radiation therapy (RT) (30% among chemotherapy recipients), although the use of RT is declining [10]. If these treatments are not effective, stem cell transplantation and the targeted drug brentuximab vedotin may be options. For patients with NLPHL, radiation alone may be appropriate for early-stage disease. For those with later-stage disease, chemotherapy plus RT as well as the monoclonal antibody rituximab may be recommended. The 5-year and 10-year survival rates for HL are 86% and 80%, respectively. The 5-year survival rate is 94% for NLPHL and 85% for CHL [7].

The most common types of NHL are Diffuse Large B-Cell Limphoma (DLBCL), representing 37% of cases, and follicular lymphoma, representing 20% of cases [8]. Although DLBCLs grow quickly, most patients with localized disease and about 50% of those with advanced-stage disease are cured [11, 12]. In contrast, follicular lymphomas tend to grow slowly and often do not require treatment until symptoms develop, but many are not curable [13]. Some cases of follicular lymphoma transform into DLBCL. The first course of treatment for all NHL subtypes combined is usually chemotherapy, either alone (58%) or in combination with RT (11%). Approximately 17% of patients receive no treatment. A monoclonal antibody like rituximab is often given along with chemotherapy for B-cell lymphomas and for some T-cell lymphomas. The 5-year survival rate is 86% for fol-

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licular lymphoma and 61% for DLBCL; 10-year survival rate declines to 77% and 53%, respectively [7].

30.2 Risk of Breast Cancer in Lymphoma Survivors

The improving of the survival in lymphoma patients using combined modalities of treatment, in which RT has a fundamental part, led to observe in this population new problems over the time, often more important than the lymphoma itself. In fact, cure has come at a price, because the treatment of lymphoma has been shown to increase the risk of subsequent malignant neoplasms and other late effects considerably [14–36]. Although very high relative risks have been observed for leukemia (especially among patients who were treated with alkylating agents) and NHL (which was not previously associated with a particular type of therapy), second solid cancers, the occurrence of which is related primarily to RT, contribute most to the absolute excess risk of a second cancer among survivors of HL. At 5–10 years after treatment, the relative risk of solid cancer is significantly higher among survivors of HL than in the general population, and this higher risk persists for at least 25 years [24, 32, 33]. Few studies have investigated the evolution of a risk of a second solid cancer beyond 25 years after treatment [20, 24, 26, 29]. On the basis of increased knowledge of late effects, the treatment of HL has changed, with a trend toward the use of smaller radiation target volumes, lower radiation doses, and more effective, generally less toxic chemotherapy schemes ([37], [38]). However, the effect of these changes on the risk of a second cancer is still unknown.

In a recent study, Schaapveld et al. [39] investigated the long-term risk of a second cancer and changes in risk over time in a large cohort of survivors of HL. The analysis evaluated 3905 persons in the Netherlands who had survived for at least 5 years after the initiation of treatment for HL. Patients had received treatment between 1965 and 2000, when they were 15–50 years of age. The risk of a second cancer among these patients was compared with the risk that was expected on the basis of cancer incidence in the general population, and treatment-specific risks were compared within the cohort. With a median follow-up of 19.1 years, 1055 second cancers were diagnosed in 908 patients, resulting in a standardized incidence ratio (SIR) of 4.6 (95% confidence interval [CI], 4.3–4.9) in the study cohort as compared with the general population. Breast cancer contributed most to the overall absolute excess risk (24.9 cases of breast cancer per 10,000 person-years among men and women), representing 20.4% of the excess risk of any second cancer (121.8 cases per 10,000 person-years) in the cohort; the absolute excess risk of breast cancer among women was 54.3 cases per 10,000 person-years, representing 40.5% of the excess risk of any second cancer (134.0 cases per 10,000 person-years) among women in the cohort. The risk was still elevated 35 years or more after treatment (SIR, 3.9; 95% CI, 2.8–5.4), and the cumulative incidence of a second cancer in the study cohort at 40 years was 48.5% (95% CI, 45.4–51.5). At 30 years, the cumulative incidence of breast cancer among women in the study cohort was 16.6% (95% CI, 14.1–19.2). The cumulative incidence of second solid cancers did not differ according to study period (1965–1976, 1977–1988, or 1989–2000) (P = 0.71 for heterogeneity) (Fig. 30.1). Although the risk of breast cancer was lower among patients who were treated with supradiaphragmatic-field RT not including the axilla than among those who were exposed to mantle-field irradiation (hazard ratio, 0.37; 95% CI, 0.19–0.72), the risk of breast cancer was not lower among patients treated in the 1989–2000 study period than among those treated in the two earlier periods. The authors concluded that the risk of second solid cancers did not appear to be lower among patients treated in the most recent calendar period studied (1989–2000) than among those treated in earlier periods.

Of course, because of the long latency required to observe second solid cancers and the rapid evolution of RT techniques, many estimates of radiation-related second cancer risk reflect outcomes of treatment no longer in use, and published risk estimates are largely based on patients treated with 35 Gy to mantle, extended-field, or subtotal nodal RT fields in the 1960s through the 1980s. Since that time, lymphoma treatment has progressed to use smaller involved-field RT fields [40, 41]. Recent clinical trial results suggest that low-dose (20 Gy) RT may emerge as standard treatment for adult lymphoma, and these new effective RT techniques probably will reduce in the future the absolute risk of secondary breast cancer expected [42–44]. The ongoing European Organization for Research and Treatment of Cancer (EORTC) H10 study aims to assess whether early assessment of response by repeat fluorodeoxyglucose positron emission tomography (FDG-PET) scans after two cycles of ABVD could help to better determine whether radiotherapy is needed in the treatment for stage I/II supradiaphragmatic Hodgkin lymphoma. This trial also introduces the concept, later published as EORTC-GELA, Groupe d'Etude des Lymphomes de l'Adulte guidelines, of initially involved "nodes" and not "field" irradiation. This concept relies on coregistered prechemotherapy and post-chemotherapy computed tomography-FDG-PET scans with intravenous contrast [45, 46].

In conclusion, improvements of RT techniques such as respiratory-gated RT and intensity-modulated RT should allow better protection of normal tissues (breast, lung, and heart) during RT for lymphoma [47]. These improvements, combined with the reduction of radiation field size (replacement of mantle-field RT by involved-field and involved-node

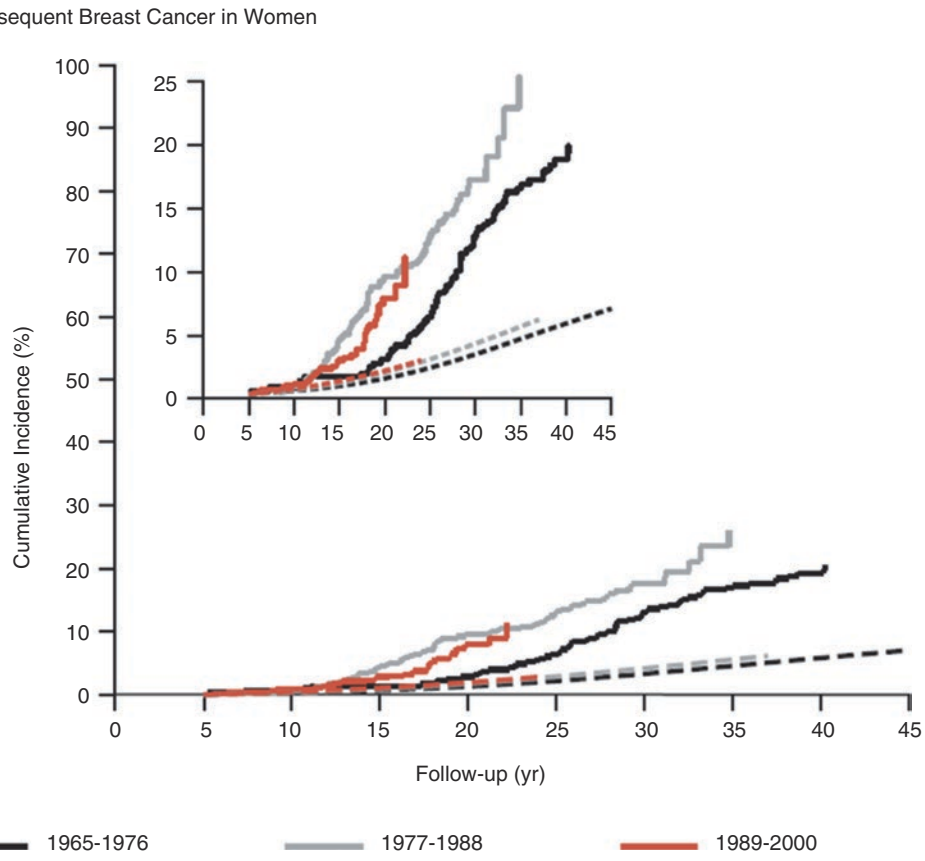

Fig. 30.1 Cumulative incidence of subsequent breast cancer in women. *Solid lines* represent the observed incidence and *dashed lines* the expected incidence in the general population. The *insets* show the same data on enlarged *y* axes (From: Second Cancer Risk Up to 40 Years after Treatment for Hodgkin lymphoma. M Schaapveld, B Aleman, AM van Eggermond. NEJM. 2015;373: 2499–511)

RT) [46], should minimize secondary breast cancer incidence in future decades.

30.3 Impact of Early Breast Cancer Screening on Mortality Among Lymphoma Survivors

Survivors of lymphoma are at increased risk for delayed morbidity related to their treatment, and increasing effort is being directed toward preventing or ameliorating late-treatment toxicity. One potentially modifiable late effect is mortality because of breast cancer among women treated with thoracic RT. In particular, adolescent females treated with thoracic RT for HL have been shown to have statistically significantly elevated risks of breast cancer [22, 48, 49], and consensus guidelines recommend the early initiation of breast cancer screening among these survivors [50]. In fact, these women must be considered as a population at very high risk of breast cancer, and, according to the age, mammary density, and menopausal status, a personalized program of breast cancer early detection should be routinely planned. Careful breast surveillance with yearly breast palpation and imaging is mandatory to provide early diagnosis of breast cancer. Unfortunately, lack of supporting evidence is a major limitation of these guidelines, however. The efficiency of screening with annual mammography in this particular population was reported in several studies [51]. Breast MRI should also help to better screen these women and was recommended as an adjunct to annual mammography for women who have been treated with radiotherapy for supradiaphragmatic HL, starting at age 25 or 8 years after completion of radiotherapy [52, 53].

A recent update of UK guidelines for the follow-up of pediatric cancer survivors, for example, could not identify a single study examining the benefits or harms of specific screening programs, including the early initiation of breast cancer screening in high-risk survivors [54]. This lack of evidence may in part account for the observation that 55–85% of eligible survivors in the US and Canada do not undergo breast cancer screening [55, 56]. While the effectiveness of early breast cancer screening should ideally be evaluated in a randomized trial, no such trial will be undertaken in the foreseeable future. To address this clinical uncertainty, Hodgson et al. [57] conducted simulations under a range of clinically plausible conditions to quantify the potential benefit of early breast cancer screening using different screening modalities among women treated with mediastinal RT for HL in adolescence. A mathematical model of breast cancer development was used to evaluate the marginal benefit of early-initiated screening of female survivors of adolescent HL starting at age 25 years on breast cancer mortality compared with

screening initiated at age 40 years. Sensitivity analyses were performed to evaluate the robustness of the estimates over a plausible range of conditions. For survivors treated at age 15 years, the absolute risk of breast cancer mortality by age 75 years was predicted to decrease from 16.65% with no early screening to 16.28% (annual mammography), 15.40% (annual MRI), 15.38% (same-day annual mammography and MRI), and 15.37% (alternating mammography and MRI every 6 months). Approximately 80 patients would need to be invited to MRI-based screening to prevent one breast cancer death. In sensitivity analyses, the number needed to invite to MRI-based screening to prevent one breast cancer death ranged from 71 to 333. Combinations of MRI plus mammography were predicted to produce 99.52 false positives per 1000 screenings done between age 25 and 39 years. The authors concluded that these findings are the first to indicate that early MRI-based screening should reduce breast cancer mortality among women treated with RT for adolescent HL. The magnitude of this benefit is superior to that described for other accepted screening indications although MRI can produce a substantial rate of false-positive results.

30.4 Breast Cancer Characteristics in Lymphoma Survivors

It is unknown whether breast cancer characteristics among young women treated with RT for lymphoma differ from sporadic breast cancer. In lymphoma survivors, there was a high rate of bilateral cancers, compared with sporadic breast cancer (from 7% to 25%) [58–60], easily explained by the symmetrical distribution of radiation dose in the two breasts during mantle-field or mediastinal RT. Clinical and pathologic characteristics of breast cancer after lymphoma have been previously detailed by several authors [58, 61–68], but only one of the above studies directly compared the characteristics and prognosis of breast cancers after lymphoma in a series of age-matched first primary breast cancer [63]. It seemed that the features and prognosis of breast cancer after lymphoma were quite similar to those of sporadic cases. In the series of the Institut Curie [64], breast cancers were diagnosed at a relatively young age (median, 43 years) compared with the general French population (median, 61 years), and overall survival and locoregional control rates seemed to be inferior to those in nonmetastatic invasive sporadic breast cancers: with a median follow-up of 7 years, the 5-year overall survival rate and locoregional control rate were, respectively, 74.5% (95% CI, 64–88%) and 82% (95% CI, 72–93.5%) for invasive carcinoma. This difference in overall survival may be partially explained by an increase in the number of deaths due to cardiovascular diseases and nonbreast cancers secondary to treatments of lymphoma. Broeks et al. [65, 66] recently suggested that radiation-associated breast cancer may be different from other tumors on the basis of their expression profile. They compared microarray-based gene expression in 22 breast cancers after HL with a set of 20 control breast cancers. Radiation-induced breast cancer seemed to be characterized by a high degree of proliferation and a more aggressive tumor type; they reported a higher chromosomal instability and higher expression of proliferation marker Ki-67 in tumors occurring in previously irradiated breasts.

Dores et al. [67] evaluated the risk of invasive breast cancer among female 5-year survivors of HL diagnosed before 35 years of age who received RT as part of initial therapy for HL and who were reported to one of nine cancer registry areas of the SEER Program in the US during 1973–2000 and followed through 2005. Compared with breast cancer in the general population, risks of estrogen receptor (ER)-positive/progesterone receptor (PR)-positive and ER-negative/PR-negative breast cancer in young, irradiated HL survivors were increased fivefold (95% confidence interval (CI) = 3.81–6.35) and ninefold (95% CI = 6.93–12.25), respectively. Among 15-year survivors, relative risk of ER-negative/PR-negative BC exceeded by twofold ($P = 0.002$) than that of ER-positive/PR-positive breast cancer. Finally, the authors concluded that RT may disproportionately contribute to the development of breast cancer with adverse prognostic features among young HL survivors.

30.5 Treatment of Breast Cancer in Lymphoma Survivors

The surgical treatment of breast cancer patients with a history of malignant lymphoma treated with RT represents a challenge. According to the American College of Radiology appropriateness criteria on conservative surgery and radiation [68], a previous history of RT (e.g., for the treatment of ML) that delivered a significant dose to the breast and for which retreatment would result in an excessively high total radiation dose to the breast tissue is a contraindication for a BCS. High radiation doses to the breast result in unacceptable long-term toxicity and poor cosmetic rates, and indeed most authors consider these patients at significant risk of complications (fibrosis, skin and soft tissue necrosis, rib fractures, potential lung and heart toxicities) [62, 69–71] and do not candidate them for BCS and adjuvant RT. The study from Stanford [62] reported one of two women, who underwent lumpectomy and breast RT, and had a severe tissue necrosis developed in her lateral breast and chest wall at the area of overlap with the previous mantle field. In contrast, other reports [58, 72–75] support BCS followed by external beam radiation when breast cancer develops many years after RT for lymphoma. So, at present, no consensus exists regarding the correct management of breast cancer after RT

for lymphoma, and, given the discordant results and the small number of women treated with BCS, mastectomy continues to be recommended as the standard treatment.

There are few studies in the literature that compare BCS and mastectomy in the setting of breast cancer patients previously treated with RT for lymphoma [64, 76, 77]. The retrospective study by Haberer et al. [64] evaluated outcomes of 72 women who developed either ductal carcinoma in situ or stage I–III invasive carcinoma of the breast after HL between 1978 and 2009 at the Institut Curie. In this large series, median age at HL diagnosis was 23 years (range, 14–53 years). Median total dose received by the mediastinum was 40 Gy, mostly by a mantle-field technique. Breast cancers occurred after a median interval of 21 years (range, 5–40 years). Ductal invasive carcinoma and ductal carcinoma in situ represented, respectively, 51 cases (71%) and 14 cases (19%). Invasive breast cancers consisted of 47 cT0–2 tumors (82%), 5 cN1–3 tumors (9%), and 20 grade 3 tumors (35%). Locoregional treatment for breast cancer consisted of mastectomy with [3] or without [36] RT in 39 patients and lumpectomy with [30] or without [2] adjuvant RT in 32 patients. The isocentric lateral decubitus radiation technique was used in 17 patients after BCS (57%). With a median follow-up of 7 years, 5-year overall survival rate and locoregional control rate were, respectively, 74.5% (95% CI, 64–88%) and 82% (95% CI, 72–93%) for invasive carcinoma and 100% (95% CI, 100–100%) and 92% (95% CI, 79–100%) for in situ carcinoma. In patients with invasive tumors, the 5-year distant disease-free survival rate was 79% (95% CI, 69–91%), and 13 patients died of progressive breast cancer. Contralateral breast cancer was diagnosed in ten patients (14%). The authors concluded that BCS can be an option for breast cancer that occur after HL, despite prior thoracic irradiation. It should consist of lumpectomy and adjuvant breast RT with use of adequate techniques, such as the lateral decubitus isocentric position, to protect the underlying heart and lung.

More recently, Cutuli et al. [78] have published a multicentric international retrospective study on 189 women previously treated for HD by RT and/or chemotherapy that subsequently developed 214 breast cancer. Median age at HD diagnosis was 25 years (34% were less than 20). Median interval between HD and breast cancer was 18.6 years, with a 42-year median age at first BC. According to the TNM classification, there were 30 (14%) T0 (non-palpable lesions), 86 (40%) T1, 56 (26%) T2, 13 (6%) T3T4, and 29 (14%) Tx. There were 25 (13.2%) contralateral breast cancers. 160 (75%) and 15 (7%) tumors were infiltrating ductal and lobular carcinomas, 7 (3.3%) were other subtypes, and 27 (22%) were DCIS. The rate of axillary nodal involvement was 32%. Among 203 operated tumors, 79 (39%) were treated by BCS, with RT in 56 (71%) cases. With a 50-month median follow-up, local recurrence occurred in 12% of the tumors (9% after mastectomy, 21% after lumpectomy alone, and 13.7% after lumpectomy with RT). Metastasis occurred in 47 (26%) patients. The risk factors were pN+, pT, high SBR grade, and young age (<50 years). The 10-year overall and specific survival rates were 53% and 63.5%, respectively. The 10-year specific survival rates were 79% for pT0T1T2, 48% for pT3T4 ($p = 0.0002$), and 79% for pN0 versus 38.5% for pN+ ($p = 0.00026$). Among 67 deaths, 43 (73%) were due to breast cancer. Comparing BCS to mastectomy group, the authors reported a similar 5- and 10-year breast cancer survival between the two groups of treatment (75.8% and 70.7% after BCS and 76.6% and 63.7% after mastectomy, respectively). No remarkable unfavorable side effects of this treatment were observed. Other small retrospective studies have been published addressing the effect of WBRT in patients who have been previously treated with RT for lymphoma and supporting the use of whole breast radiation therapy (WBRT) after breast-conserving surgery (BCS) as re-irradiation with limited toxicity and good tolerance, especially in case of long interval of time between lymphoma and breast cancer [62, 73, 79–82]. Deutsch et al. [73] reported 12 patients with breast cancer developing after previous RT for HD treated by BCS followed by WBRT. With a median follow-up of 46 months, the authors reported two deaths due to distant disease. No patients developed local recurrence, and 100% of patients had excellent cosmetic results.

To avoid the risk of cumulative doses of RT to the thoracic wall, reason why total mastectomy has been historically considered the standard of therapy for these patients, especially in case of young patients and short interval between lymphoma and breast cancer diagnosis [83], one of the conservative options is to treat just the tumor bed. The irradiation of a small volume of the breast and adjacent structures could minimize the risk of complications [84–92]. Advances in RT devices and the development of different forms of partial breast irradiation (PBI) techniques, such as interstitial or intracavitary brachytherapy (MammoSite applicator), intraoperative radiotherapy with electrons (IOERT), intraoperative orthovoltage device (Intrabeam), and 3-D conformal or intensity-modulated external beam RT, could represent safe alternatives to avoid unnecessary mastectomies and provide a breast-conserving option for these patients, improving the local control of the disease. All of these techniques have similar indications but different applications. In particular, they differ in the source of radiation (e.g., X-ray, iridium-192, photons) and the amount of breast volume treated [93–101]. Data from recent reports consider PBI as a real alternative to mastectomy for these patients, given that the irradiation of a small volume of the breast minimizes the risk of complications, and report excellent outcomes and local control in carefully selected patients. An early series by Chadha et al. [102] examined the feasibility of partial breast brachytherapy as the primary treatment for breast cancer diagnosed after mantle

RT for HL. Although the study included a very specific group of five BC patients (<1 cm), no evidence of recurrence was reported. Moreover, the reported toxicity never exceeded grade 2, and cosmetic outcome was reported as excellent in all patients with no severe late sequelae from the repeat course of RT.

In a previous report of our group, Intra et al. [103] performed a retrospective review to assess the potential of performing BCS and intraoperative radiotherapy with electrons (IOERT) in 43 patients affected by early breast cancer, previously treated with mantle radiation for lymphoma. Median age at diagnosis of lymphoma was 26 years (49% were less than 25). Median interval between lymphoma and breast cancer occurrence was 19 years. A total dose of 21 Gy (prescribed at 90% isodose) in 39 patients (91%), 17 Gy (prescribed at 100% isodose) in 1 patient, and 18 Gy (prescribed at 90% isodose) was delivered. IOERT was well tolerated in all patients without any unusual acute or late reactions. After a median follow-up of 52 months, local recurrence occurred in 9% of the patients and metastases in 7% patients. In this series, intraoperative technique offered the advantage of a very precise targeting of the tumor bed, sparing normal breast tissue and surrounding vital organs from a second course of irradiation. IOERT allowed a breast conservative treatment in selected breast cancer patients, independently of the interval between mantle RT and surgery, without acute, intermediate, and late side effects, so decreasing the number of avoidable mastectomies and achieving a good local control of the disease. Unfortunately, no reports have been published yet comparing PBI techniques and WBRT as re-irradiation therapy during BCS in patients with history of RT for lymphoma.

30.6 Treatment of Breast Cancer in Lymphoma Survivors at the European Institute of Oncology (EIO)

In order to evaluate the long-term survival outcomes with BCS and mastectomy in the management of breast cancer patients with a history of lymphoma treated with RT and the role of WBRT and PBI during BCS from a total number of 155 lymphoma survivor patients who developed breast cancer and were treated at the EIO from September 1994 to December 2012, we retrospectively reviewed the medical records of 113 consecutive patients diagnosed with operable breast cancer previously submitted to RT for lymphoma. Thirty-two patients were excluded from the analysis given that no history of RT for lymphoma was retrieved. We excluded patients diagnosed with intraductal carcinoma (DCIS) or inflammatory breast cancer and those with concomitant diagnosis of lymphoma and breast cancer. We also excluded patients with clinical evidence of metastatic disease at the time of breast cancer diagnosis.

30.6.1 Malignant Lymphoma Data

For the entire cohort, lymphoma characteristics and treatment modalities were extracted from each patient's medical record. Type of lymphoma, age at diagnosis, interval time between lymphoma diagnosis and the occurrence of breast cancer, and the percentage of patients treated with chemotherapeutic agents were obtained. Detailed information of RT fields used on lymphoma treatment was also collected: mantle-field radiation included the primary lymph node regions of the neck, supraclavicular, infraclavicular, axillary, and mediastinal areas. Other RT fields included the involved supradiaphragmatic lymph node site or the involved supradiaphragmatic lymph node.

30.6.2 Breast Cancer Data

Clinical data collected included type of surgery, age at breast cancer diagnosis, percentage of contralateral breast cancer, and adjuvant therapy received. Breast surgical treatment consisted of either quadrantectomy ± RT or mastectomy followed or not by postoperative RT. Axillary staging was performed using sentinel lymph node (SLN) biopsy followed by axillary lymph node dissection (ALND) in case of macrometastatic SLN or primary axillary clearance in patients with preoperative assessment of axillary involvement. According to our policy at that time, in case of isolated tumor cells (ITCs) or micrometastasis of SLN, axillary dissection was omitted. Re-irradiation was administered after or during surgery by either whole breast radiation therapy (WBRT) or intraoperative radiotherapy with electrons (IOERT). WBRT consisted of a classical course of postoperative fractionated irradiation of the entire breast followed by a boost on the tumor bed. IOERT during BCS was delivered in a full-dose course directly to the tumor bed during the intervention, according to our standardized technique previously described [104].

Pathological data was retrieved directly from the pathological reports and include histological subtype, size of tumor (T), number of positive lymph nodes (N), estrogen/progesterone receptor status (ER, PgR), Her-2/neu status, proliferative index (Ki-67), nuclear grade (G), and presence or absence of perivascular invasion (PVI).

Patients were followed up with physical examination with blood tests every 6 months, breast and axillary ultrasound every 6 months, annual mammography, and further systemic evaluations only in case of symptoms. When possible, the status of women not presenting at the institute for scheduled follow-up visits for more than 1 year was obtained

by telephone contact. From follow-up data, we identified local and axillary failures, contralateral breast tumor occurrence, distant metastasis, and death. To evaluate acute and late radiation morbidity, the Radiation Therapy Oncology Group (RTOG) and European Organization for Research and Treatment of Cancer (EORTC) scoring scheme ([105]) was applied on the first and seventh day of the radiosurgical treatment and every 6 months during follow-up.

30.6.3 Statistical Analysis

Differences in the distribution of subject characteristics between patients receiving BCS and patients receiving mastectomy were evaluated by the chi-square test. The end points evaluated were disease-free survival (DFS), overall survival (OS), cumulative incidence of locoregional recurrence (CI-LR), and distant metastasis (CI-DM), all measured from the date of surgery. DFS was defined as the time from surgery to events such as relapse (including ipsilateral breast recurrence), appearance of a second primary cancer (including contralateral breast cancer), or death, whichever occurred first. OS was defined as the time from surgery until the date of death (from any cause). The DFS and OS functions were estimated using the Kaplan–Meier method. The log-rank test was used to assess differences between groups. The CI-LR and CI-DM were defined as the time from the date of surgery to a locoregional recurrence and a distant metastasis, respectively. The CI-LR and CI-DM curves functions were estimated according to methods described by Kalbfleisch and Prentice [106], taking into account the competing causes of recurrence. The Gray's test was used to assess cumulative incidence differences between groups [107]. All analyses were carried out with the SAS software (SAS Institute, Cary, NC) and the R software (http://cran.r-project.org/) with the cmprsk package developed by Gray (http://biowww.dfci.harvard.edu/_gray). All reported p values are two-sided.

30.6.4 Patient Population and Tumor-Related Characteristics

A total of 113 patients were included in this analysis. Demographic and clinical characteristics of the groups according to type of surgery are shown in Table 30.1. Mastectomy was treatment of choice in 36 patients while BCS was performed in 77. In 70 patients out of 77, some type of RT was added during or after BCS. In particular, WBRT was performed in 18 patients while IOERT in 52. WBRT consisted of 50 Gy administered in 5 weeks followed by a boost of 10 Gy on the tumor bed in all the patients. In case of IEORT, we treated two patients with a total dose of 17 Gy (prescribed at 100% isodose) and three patients with 18 Gy (prescribed at 90% isodose). The remaining 47 patients (91%) received 21 Gy (prescribed at 90% isodose). The median interval time between diagnosis of lymphoma and the occurrence of breast cancer was 19 years (range 1–46 years). Median age at diagnosis of lymphoma was 25 years (range 11–66 years) and 46 years (range 29–73 years) at breast cancer diagnosis. As expected, there were significant differences between the BCS and mastectomy cohorts regarding tumor size ($p < 0.001$), nodal status ($p = 0.003$), expression of Her2 oncoprotein ($p = 0.046$), PVI ($p = 0.028$), and nuclear grade ($p = 0.031$) (Table 30.2). Median follow-up for the entire population was 7.3 years (range: 6 months to 18 years).

30.6.5 Survival Outcomes for the Entire Population

Although both groups of treatment were not comparable because of heterogeneity, a comparative analysis of survival outcomes was performed (Table 30.3). The 5- and 10-year OS rates were 94% and 86.7%, respectively, in the BCS group vs. 74.1% and 68%, in the mastectomy group. Log-rank tests indicated significantly different survival curves at the 5- and 10-year points ($p = 0.022$) (Fig. 30.2a). The 5-year disease-free survival rates for patients who underwent BCS and mastectomy were 79.2% (95% CI 67.2%–87.2%) and 57.9% (36.4%–74.4%), respectively. The 10-year DFS rates for patients treated with BCS and mastectomy were 65.9% (50.3%–77.6%) and 45.6% (23.8%–65.1%), respectively. Log-rank tests indicated significantly different survival curves at the 5- and 10-year points ($p = 0.039$) (Fig. 30.2b).

The number of observed events according to primary local treatment is shown in Table 30.4. In the total population, 12 patients developed locoregional recurrence (LRR) (10.6%). Eleven patients had a LRR (14.2%) in the BCS group and 1 (2.7%) in the mastectomy group. Local recurrence (LR) as first event occurred in 11 patients (91.6%), while 1 patient (8.3%) developed regional recurrence (RR). There was no significant difference in the cumulative incidence of locoregional recurrence events among BCS and mastectomy groups ($p = 0.124$). Distant metastasis occurred in ten patients, four of whom were treated with BCS and six patients received a mastectomy. The 10-year cumulative incidence of distant metastasis was 5.2 (95% CI 1.7–16.1) for BCS and 20.5% (95% CI 9.7–43.2) for patients treated with mastectomy ($p = 0.015$). Overall, 19 of 113 patients (16.8%) died, 13 due to BC (19.4 in the mastectomy group and 7.8% in the BCS group), 2 due to other tumors, and 4 from other/unknown causes. No several early or late side effects were reported.

Table 30.1 Demographic and clinical characteristics according to primary local treatment

	All patients	BCS	Mastectomy	
	N (% col)	*N*(% col)	*N* (% col)	*P* value
	113 (100)	77 (100)	36 (100)	
Type of lymphoma				0.095
Hodgkin	90 (79.6)	58 (75.3)	32 (88.9)	
Non-Hodgkin	23 (20.4)	19 (24.7)	4 (11.1)	
Age at diagnosis of lymphoma				0.173
<20	37 (32.7)	21 (27.3)	16 (44.4)	
20–30	42 (37.2)	30 (39.0)	12 (33.3)	
>30	34 (30.1)	26 (33.8)	8 (22.2)	
Age at breast cancer diagnosis				0.274
<35	14 (12.4)	7 (9.1)	7 (19.4)	
35–39	12 (10.6)	9 (11.7)	3 (8.3)	
40–49	44 (38.9)	28 (36.4)	16 (44.4)	
50–59	24 (21.2)	17 (22.1)	7 (19.4)	
≥60	19 (16.8)	16 (20.8)	3 (8.3)	
Years between lymphoma and breast cancer diagnosis				**0.033**
<10	20 (17.7)	17 (22.1)	3 (8.3)	
10–19	37 (32.7)	24 (31.2)	13 (36.1)	
20–29	40 (35.4)	22 (28.6)	18 (50)	
≥30	16 (14.2)	14 (18.2)	2 (5.6)	
Radiotherapy for lymphoma				**0.022**
Mantle field	67 (59.3)	39 (50.6)	28 (77.8)	
Other	46 (40.7)	38 (49.4)	8 (22.2)	
Chemotherapy for lymphoma				0.264
No	30 (26.5)	18 (23.4)	12 (33.3)	
Yes	83 (73.5)	59 (76.6)	24 (66.7)	
Radiotherapy for BC				**<0.001**
No	34 (30.1)	7 (9.1)	27 (75)	
Yes—WBRT	20 (17.7)	18 (23.4)	2 (5.6)	
Yes—IOERT	59 (52.2)	52 (67.5)	7 (19.4)	
Adjuvant systemic therapy for BC				0.024
No	7 (6.2)	3 (3.9)	4 (11.1)	
Hormonotherapy	67 (59.3)	53 (68.8)	14 (38.9)	
Chemotherapy	22 (19.5)	12 (15.6)	10 (27.8)	
Hormono-chemotherapy	17 (15)	9 (11.7)	8 (22.2)	
Neoadjuvant chemotherapy for BC				0.055
No	98 (86.7)	70 (90.9)	28 (77.8)	
Yes	15 (13.3)	7 (9.1)	8 (22.2)	

Abbreviations: *BCS* breast-conserving surgery, *BC* breast cancer, *WBRT* whole breast radiation therapy, *IOERT* intraoperative radiotherapy with electrons. Bold print indicates a *p* value <0.05

30.6.6 Survival Outcomes Comparing BCS Plus Re-irradiation Versus Mastectomy in Patients with T1 Tumor

A subgroup evaluation on 73 patients with tumors smaller than 2 cm (T1) who had not been previously treated with neoadjuvant chemotherapy was performed. The majority of patients were treated with BCS ($n = 57$) plus RT and the others were treated with mastectomy ($n = 16$). A comparative analysis between the two groups of patients did not show any statistically significant difference in terms of OS ($p = 0.5$), DFS ($p = 0.25$), cumulative incidence of locoregional events ($p = 0.835$), and distant metastasis ($p = 0.359$).

30.6.7 Survival Outcomes According to Type of Radiation Therapy After BCS

We also performed an analysis of survival outcomes for patients undergoing BCS plus RT, comparing WBRT

Table 30.2 Histopathological characteristics of breast cancer according to type of surgery

	All patients	BCS	Mastectomy	
	N (% col)	*N* (% col)	*N* (% col)	*P* value
	113 (100)	77 (100)	36 (100)	
Histology				0.695
Ductal	99 (87.6)	67 (87.0)	32 (88.9)	
Lobular	8 (7.1)	5 (6.5)	3 (8.3)	
Other	6 (5.3)	5 (6.5)	1 (2.8)	
pT				**<0.001**
X	6 (5.3)	2 (2.6)	4 (11.1)	
1	81 (71.7)	68 (88.3)	13 (36.1)	
2	23 (20.4)	7 (9.1)	16 (44.4)	
3	3 (2.7)	0 (0)	3 (8.3)	
Number of positive lymph nodes				**0.003**
pNX	2 (1.8)	1 (1.3)	1 (2.8)	
None	66 (58.4)	52 (67.5)	14 (38.9)	
1–3	30 (26.5)	20 (26)	10 (27.8)	
4–9	12 (10.6)	3 (3.9)	9 (25)	
10+	3 (2.7)	1 (1.3)	2 (5.6)	
ER expression (%)				0.222
Unknown	5 (4.4)	2 (2.6)	3 (8.3)	
0	23 (20.4)	13 (16.9)	10 (27.8)	
1–49	2 (1.8)	2 (2.6)	0 (0)	
50–100	83 (73.5)	60 (77.9)	23 (63.9)	
PgR expression (%)				**0.006**
Unknown	5 (4.4)	2 (2.6)	3 (8.3)	
0	31 (27.4)	16 (20.8)	15 (41.7)	
1–49	27 (23.9)	17 (22.1)	10 (27.8)	
50–100	50 (44.2)	42 (54.5)	8 (22.2)	
Ki-67 expression (%)				0.264
Unknown	6 (5.3)	3 (3.9)	3 (8.3)	
<14%	34 (30.1)	26 (33.8)	8 (22.2)	
> = 14%	73 (64.6)	48 (62.3)	25 (69.4)	
Her2 status				**0.046**
Unknown	17 (15)	10 (13)	7 (19.4)	
Negative	83 (73.5)	61 (79.2)	22 (61.1)	
Positive	13 (11.5)	6 (7.8)	7 (19.4)	
Perivascular invasion				**0.028**
Unknown	**4 (3.5)**	**2 (2.6)**	**2 (5.6)**	
Absent	82 (72.6)	61 (79.2)	21 (58.3)	
Present	27 (23.9)	14 (18.2)	13 (36.1)	
Grade				**0.031**
Unknown	15 (13.3)	8 (10.4)	7 (19.4)	
1–2	60 (53.1)	47 (61)	13 (36.1)	
3	38 (33.6)	22 (28.6)	16 (44.4)	

Abbreviations: *BCS* breast-conserving surgery, *N* number of patients, *T* tumor size according to TNM classification, *N* lymph node, *ER* estrogen receptor, *PgR* progesterone receptor. Bold print indicates a *p* value <0.05

(18 patients) and IOERT (52 patients) (Fig. 30.3). OS and DFS did not differ between the IOERT group and WBRT group ($p = 0.829$ and $p = 0.35$, respectively). There were no significant differences between the two groups with regard to cumulative incidence of local recurrence or distant metastasis ($p = 0.165$ and $p = 0.576$, respectively) (Fig. 30.4).

30.6.8 Radiation Morbidity and Cosmetic Outcome in BCS Group

No increased postoperative complications (pain, seroma, hematoma, infection) were observed in patients submitted to BCS and RT (WBRT or IOERT). The length of hospital stay

Table 30.3 Survival outcomes by surgical treatment groups

	BCS	Mastectomy	All patients
	77	36	113
Overall survival (OS)			
5-year OS (95% CI)	94 (84.9–97.7)	74.1 (52.7–86.9)	88.3 (79.8–93.4)
10-year OS (95% CI)	86.7 (73–93.8)	68 (44.7–83.1)	81.3 (70.1–88.6)
P log-rank test	**0.022**		
Disease-free survival (DFS)			
5-year DFS (95% CI)	79.2 (67.2–87.2)	57.9 (36.4–74.4)	73 (62.6–80.9)
10-year DFS (95% CI)	65.9 (50.3–77.6)	45.6 (23.8–65.1)	60.1 (47.4–70.6)
P log-rank test	**0.039**		
Cumulative incidence of LR events			
5-year (95% CI)	10.7 (5.3–21.8)	5.3 (0.8–35.1)	8.9 (4.6–17.5)
10-year (95% CI)	19.9 (10.9–36.3)	5.3 (0.8–35.1)	15.6 (8.7–28.1)
P Gray test	0.124		
Cumulative incidence of DM			
5-year (95% CI)	2.9 (0.7–11.4)	20.5 (9.7–43.2)	8.2 (4.2–16)
10-year (95% CI)	5.2 (1.7–16.1)	20.5 (9.7–43.2)	9.8 (5.2–18.6)
P Gray test	**0.015**		

Abbreviations: *BCS* breast-conserving surgery, *LR* locoregional, *DM* distant metastasis. Bold print indicates a p value <0.05

was therefore not prolonged. The cosmetic outcome was also very good in all patients. In an analysis comparing BCS plus WBRT or IORT, no differences were found in terms of late side effects related to RT in both groups of patients.

In conclusion, early breast cancer patients previously irradiated for lymphoma have similar survival outcomes independently if treated with BCS or mastectomy. Breast re-irradiation after BCS can improve the local control of the disease and doesn't increase local toxicity. In selected patients, IOERT can be a valid alternative to WBRT, without acute, intermediate, and late side effects and with an acceptable cosmetic outcome. A thorough discussion with the patient is needed, and the patient's preference should be carefully taken into account after receiving all the available information on the different treatment options.

Conclusions

The treatment of malignant lymphoma has improved over the past decades: more than 90% of patients with localized lymphoma are cured with modern therapies combining chemotherapy and RT. With improved survival rates, long-term toxicities, especially increased incidence of second malignancies, are a major concern for survivors. Several studies have reported evidence that female survivors of lymphoma treated with RT are at increased risk for breast cancer, especially women treated before the age of 20 years. In women with a history of malignant lymphoma, breast cancer is the most common second malignancy, with a standardized incidence ratio of approximately six compared with the general population. The cumulative incidence of breast cancer by the age of 40–45 years ranges, in these women, from 13% to 20%. Risk factors for subsequent breast cancer after lymphoma have been described and discussed by many authors: splenectomy, young age at supradiaphragmatic irradiation, long interval after lymphoma, higher dose and volume of irradiation, and chemotherapy. Only few data are available, however, regarding locoregional treatment for breast cancer occurring after lymphoma. Mastectomy alone is the standard treatment because a breast-conserving option would necessarily entail re-irradiation of tissues already exposed to a mantle or mediastinal RT field and therefore expose the patient to potentially high cumulative doses. Breast-conserving treatment can be an option for breast cancers that occur after lymphoma, despite prior thoracic irradiation. It should consist of tumor resection and adjuvant breast RT with use of adequate techniques, such as the lateral decubitus isocentric position, to protect the underlying heart and lung. In selected patients, PBI can be a valid alternative to whole breast RT, without acute, intermediate, and late side effects and with good cosmetic results. A thorough discussion with the patient is needed, and the patient's preference should be carefully taken into account after receiving all the available information on the different treatment options. In fact, for patients with newly diagnosed lymphoma, the risks of both radiation-related and chemotherapy-related late toxic effects must be carefully balanced against the risk of failing to control the primary disease. Awareness of the increased risk of subsequent malignant neoplasms remains of great importance for survivors of lymphoma and for their physicians.

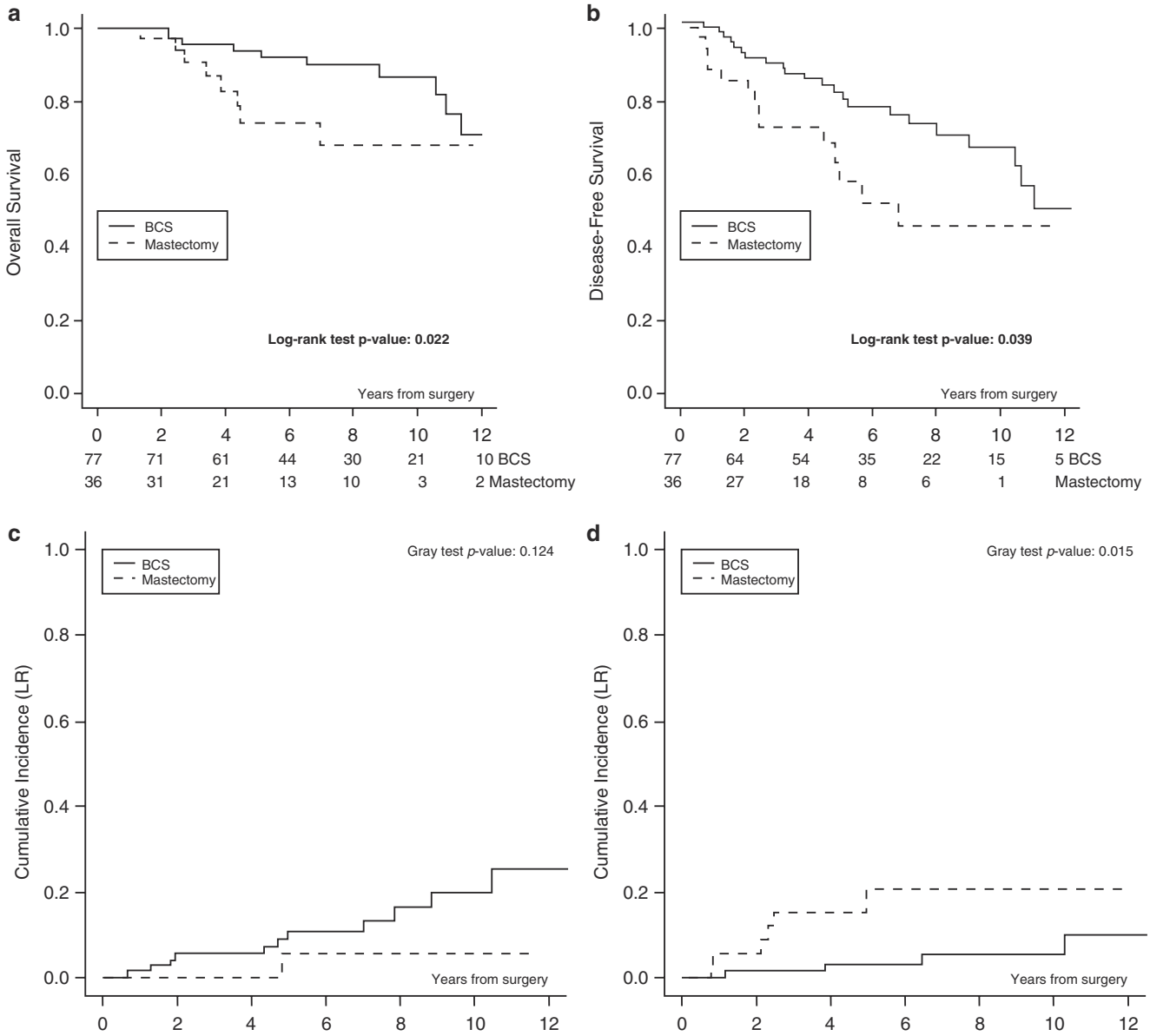

Fig. 30.2 Survival outcomes comparing breast-conserving surgery (BCS) and mastectomy for the entire cohort of patients. Overall survival (**a**) and disease-free survival (**b**) for BCS and mastectomy. Cumulative incidence estimates for locoregional recurrence (**c**) and distant metastasis (**d**) by type of local treatment

Table 30.4 Observed deaths and events according to type of surgery for the entire cohort

	BCS	Mastectomy	All patients
	77	36	113
Observed deaths, no. (%)	10 (13.0)	9 (25.0)	19 (16.8)
Breast related, no.	6	7	13
Other tumors, no.	1	1	2
Other/unknown causes, no.	3	1	4
Observed events, no. (%)	22 (28.6)	14 (38.9)	36 (31.9)
Local recurrence, no.	10	1	11
Axillary recurrence, no.	1	0	1
Contralateral, no.	3	3	6
Distant metastases, no.	4	6	10
Other events, no.	4	4	8

Abbreviations: BCS breast-conserving surgery. Bold print indicates a *p* value <0.05

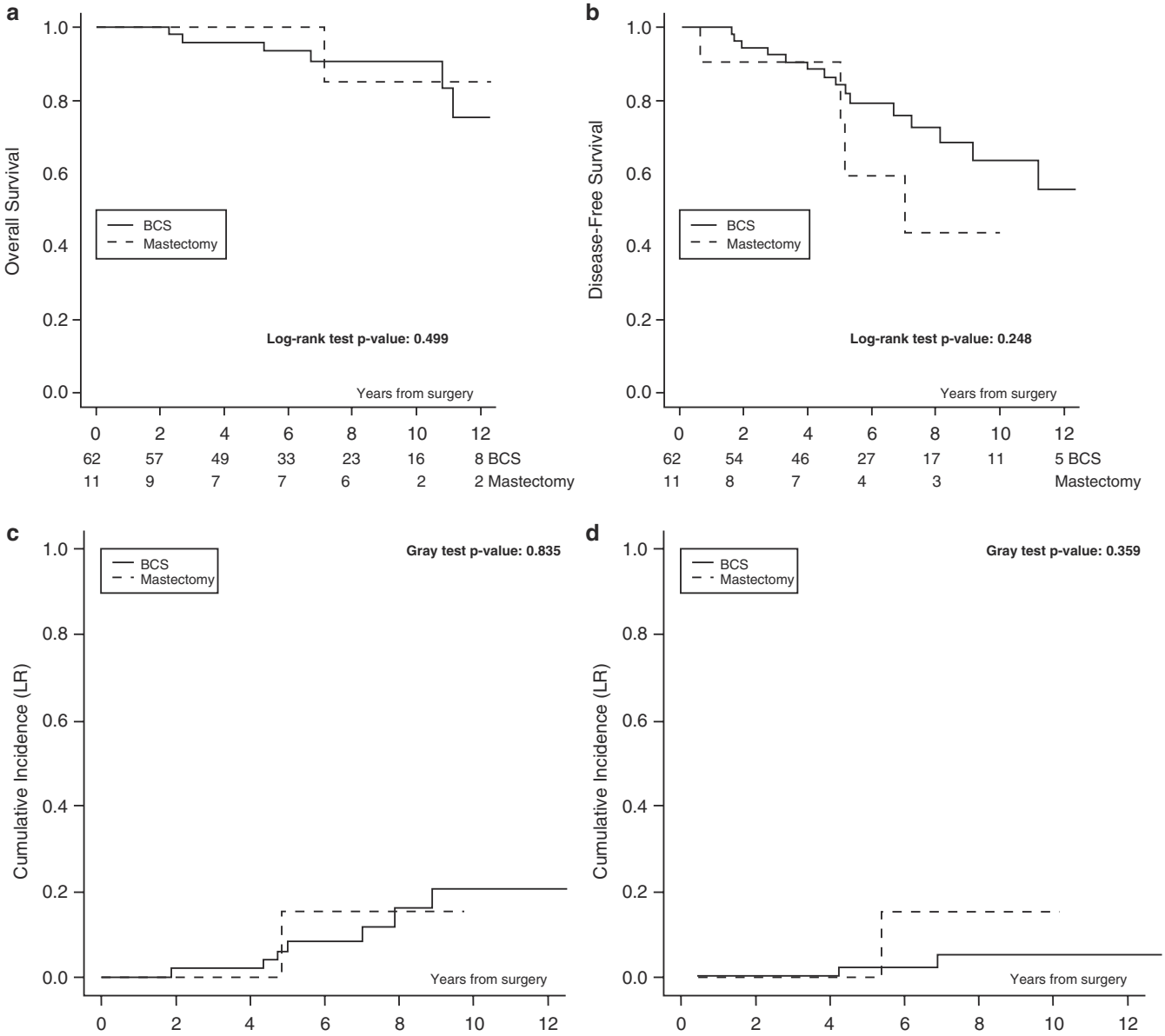

Fig. 30.3 Survival outcomes comparing breast-conserving surgery (BCS) and mastectomy for patients with tumors smaller than 2 cm (only T1 patients). Kaplan–Meier overall survival (**a**) and disease-free survival (**b**) for BCS and mastectomy. Cumulative incidence estimates for locoregional recurrence (**c**) and distant metastasis (**d**) by type of local treatment

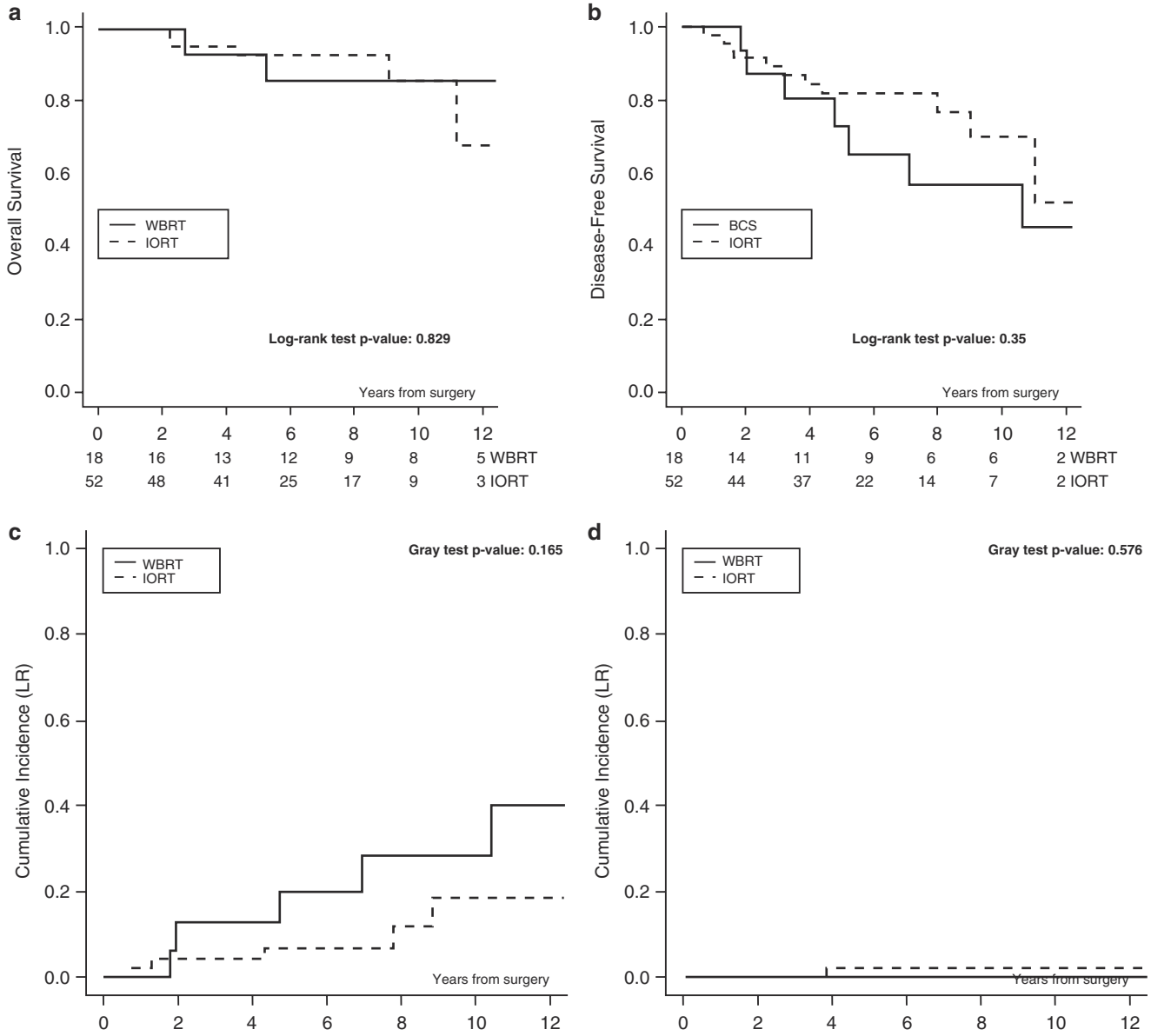

Fig. 30.4 Survival outcomes comparing breast-conserving surgery (BCS) plus whole breast radiation therapy (WBRT) and BCS plus intraoperative radiotherapy with electrons (IOERT). Kaplan–Meier overall survival (**a**) and disease-free survival (**b**) for BCS plus WBRT and IOERT. Cumulative incidence estimates for locoregional recurrence (**c**) and distant metastasis (**d**) by type of radiation therapy

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Surgery of Metastases in Stage IV Breast Cancer

31

Elisabetta Pennacchioli, Gianluca Varano, Franco Orsi, Pierpaolo Prestianni, Gianmarco Orsolini, and Angela Cioffi

31.1 Introduction

Breast cancer is the most common cancer among women worldwide.

Mortality rates have been decreasing since the 1990s, as a result of earlier detection through screening, increasing awareness, as well as advances in adjuvant therapies. Nonetheless, 3–4% of patients show synchronous metastasis at time of diagnosis [1–3].

As a result, over three million women in the United States live with a history of breast cancer, representing 41% of all female cancer survivors [4] and 3.6% of the whole American population [4, 5].

In Italy, about 50,800 new cases are recorded every year (AIRTUM, Italian Association of Tumor Registry, 2015).

The prognosis of stage IV breast cancer patients is poor with a median survival of 18–24 months, but has improved due to advances in systemic therapy. Surveillance, Epidemiology, and End Results (SEER) analysis data supports the concept that surgery may contribute to a better outcome advantage by reducing the burden of disease [6].

Patients with a higher overall survival tend to be younger females with an excellent performance status and a limited metastatic disease, for whom an intensified multidisciplinary approach combining systemic therapies with local treatment may prevent local complications and prolong survival [7, 8].

Metastatic disease is normally treated with systemic therapy, but sometimes surgery may play a role in symptom management, on a case-by-case basis. Recurrences, when they do occur, normally do so within the first 5 years, particularly in hormone receptor-negative or human epidermal growth factor 2 (HER-2)-positive disease, but sometimes they tend to be more indolent (hormone receptor-positive or HER-2-negative disease) [9].

31.2 Selection of Patients

The selection of candidates for surgery requires a careful assessment of medical conditions, extent and clinical behavior of the disease, and feasibility of resecting the metastasis with a negative margin.

The relative risks and benefits of surgery must be weighed for each individual patient.

Predictive factors are described in Table 31.1, with identification of different determinants related to the patients and to the disease.

31.2.1 Clinical Determinants

- Static clinical determinants:
  - Performance status, comorbidity
  - Extent of disease (life-threatening organs, brain mets)
  - Tumor burden
- Dynamic determinants (before and during therapy):
  - Kinetics of the disease
  - Time to progression
  - Disease aggressivity

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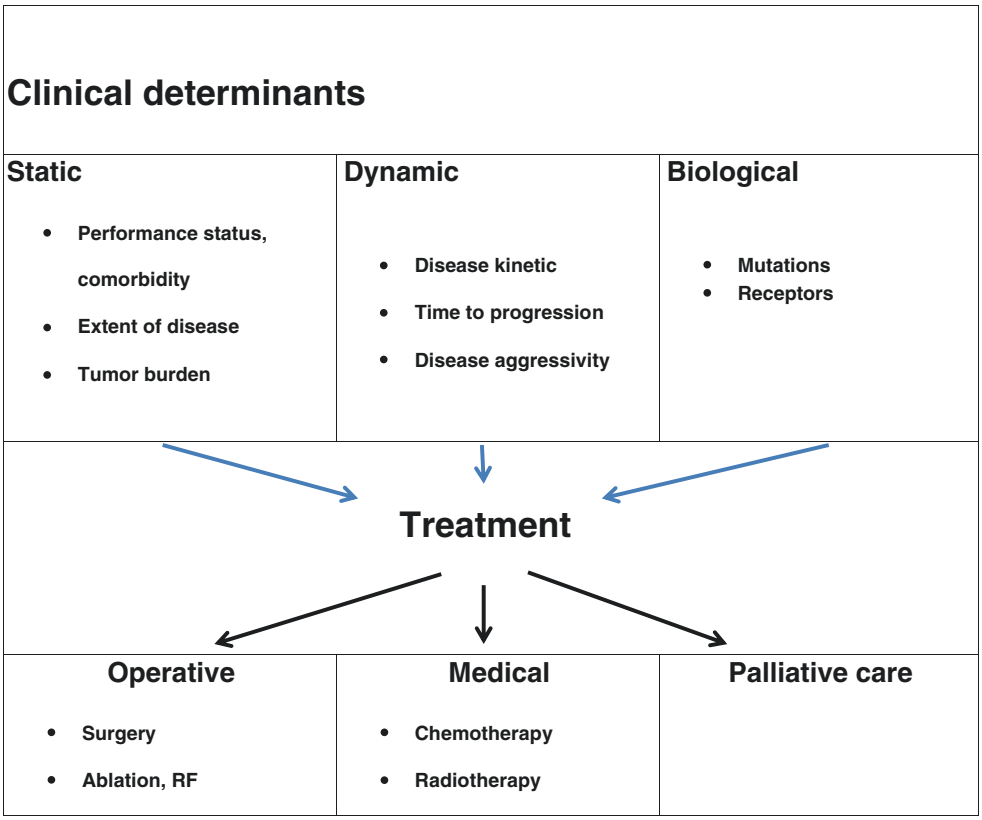
Table 31.1 Determinants which may help the selection of appropriate patients for surgical consideration



31.2.2 Biological Determinants

- Receptors
- Mutations

31.2.3 Treatment Strategy

- In fast disease kinetics (i.e., immediate danger), the first objective is to preserve life and relieve symptoms in the short term:
  - Fast-acting treatment.
  - Long-term survival is only a secondary objective.
- In intermediate disease kinetics, the first objective is to prolong survival:
  - The ideal strategy is to start with the treatment that will give the highest chance of a prolonged survival (3–5 years).
- In slow disease kinetics, the first objective is to prolong survival with the best possible quality of life:
  - The current strategy is to start with treatments such as surgery, radiosurgery, low toxicity, low morbidity, and some potential to preserve long-term survival.

31.2.4 Performance Status and Comorbidity

Perhaps the most important issues in choosing patients for local treatment of metastatic disease are the performance status and the estimation of the relative risks of a planned operation. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or worse or those with significant medical comorbidity should be considered carefully for local treatment, especially surgery, as they are likely to have high rates of postoperative morbidity and mortality.

31.2.5 Extent of Disease

Overall indicated as oligometastatic disease, disease extent is an important parameter which considers not only the number of metastatic sites but also the location in a single or multiple organs.

Multivariate analysis of local treatment outcomes suggests that those patients with limited metastatic disease, i.e., solitary or few detectable lesions, and limited to a single organ are more likely to benefit from local therapy than those with multiple metastases [10–14].

A small number of studies have reported no difference in long-term outcomes in patients undergoing hepatic resection in the presence or absence of extrahepatic disease (predominantly the bone) [15–17].

Successful surgery for metastatic disease is limited to patients with a single-organ involvement, as reported in some studies, even though there is a lack of experience in multi-organ resected patients.

31.2.6 Disease-Free Interval

A long disease-free interval (DFI) is associated with a better outcome with local therapy [18–20].

The specific cutoff value of DFI that best discriminates between favorable and unfavorable outcomes is unclear.

31.2.7 Complete Resection

Breast cancer has the potential to metastasize to almost every organ in the body. The most common sites of metastases are the bone, liver, and lung, while 15–40% of recurrences involve the chest wall and axillary or supraclavicular lymph nodes.

Approximately 50–75% of patients relapse distantly in a single organ, while the rest develop diffuse metastatic disease.

Fewer than 5% of patients manifest central nervous system (CNS) involvement as the first site of metastatic disease.

A careful preoperative evaluation is necessary to determine the feasibility of a complete resection of the metastatic deposit. The impact of positive margins is unclear, even though in some series they are associated with worse outcomes [10, 18, 20–22].

Surgical resection should be proposed when can be margin-free, alone, or in association with other means of local therapy (such as radiation therapy, radiofrequency ablation, or cryotherapy).

Several small studies have shown that treatment of isolated metastases is associated with a better progression-free survival and overall survival than oligometastatic cases.

However, with the exception of brain and spinal cord metastases, there are no prospective randomized clinical trials to determine whether or not such approaches really improve palliation and/or survival.

31.2.8 Resection of the Primary Tumor in Stage IV Breast Cancer

Management of the intact primary tumor in women presenting a stage IV breast cancer has classically been determined by the presence or absence of symptoms. However, multiple retrospective reviews suggest a survival advantage with resection of the intact, asymptomatic primary tumor in these cases [23]. Recently completed randomized trials (NCT00193778 [India] and NCT00557986 [Turkey]) do not support a significant survival benefit, although local control benefits may exist. The biases of the retrospective data include the use of surgery in younger women with smaller tumors, single sites of metastasis, and less visceral disease. Timing of surgery in relation to the diagnosis of metastases and use of systemic therapy has not always been specified in the published retrospective literature, although several authors have attempted to address this matter with varying conclusions [24–26].

Until additional unbiased data are available, surgery and/or radiotherapy should not be routinely recommended for patients with stage IV breast cancer with an intact primary tumor.

In particular, there is no basis for recommending surgery to women with distant disease if:

- The distant disease is not well controlled, and survival will likely not be long enough for the primary site to become a problem.
- Both local and distant sites are well controlled, in which case the primary site is likely to remain well controlled for the patient's life span.

The only possible exception to these general rules may be the patient with well-controlled or ablated oligometastatic distant disease, who would be rendered stage IV NED (no evidence of disease) through resection of the primary tumor.

For the patient whose distant disease is controlled but whose primary site is progressing, surgery provides a reasonable approach, although whether radiotherapy following surgery is beneficial is unproven; its use may be justified if the risk is high of early local recurrence and uncontrolled chest wall disease.

Locoregional therapy for the primary tumor should be offered to patients only with full disclosure of the lack of evidence of a survival benefit.

31.2.9 Lung Metastases

Isolated lung metastases occur in 10–25% of patients with metastatic breast cancer [11, 19] (Fig. 31.1). While pulmonary metastasectomy is a commonly performed operation, belief in its effectiveness is based on tumor registry data and surgical follow-up studies.

The necessity to rule out lung cancer in a breast cancer patient presenting with pulmonary nodes is clear; pulmonary resection may be diagnostic as well as therapeutic, since a significant number of solitary pulmonary nodules are primitive lung cancer [27–30].

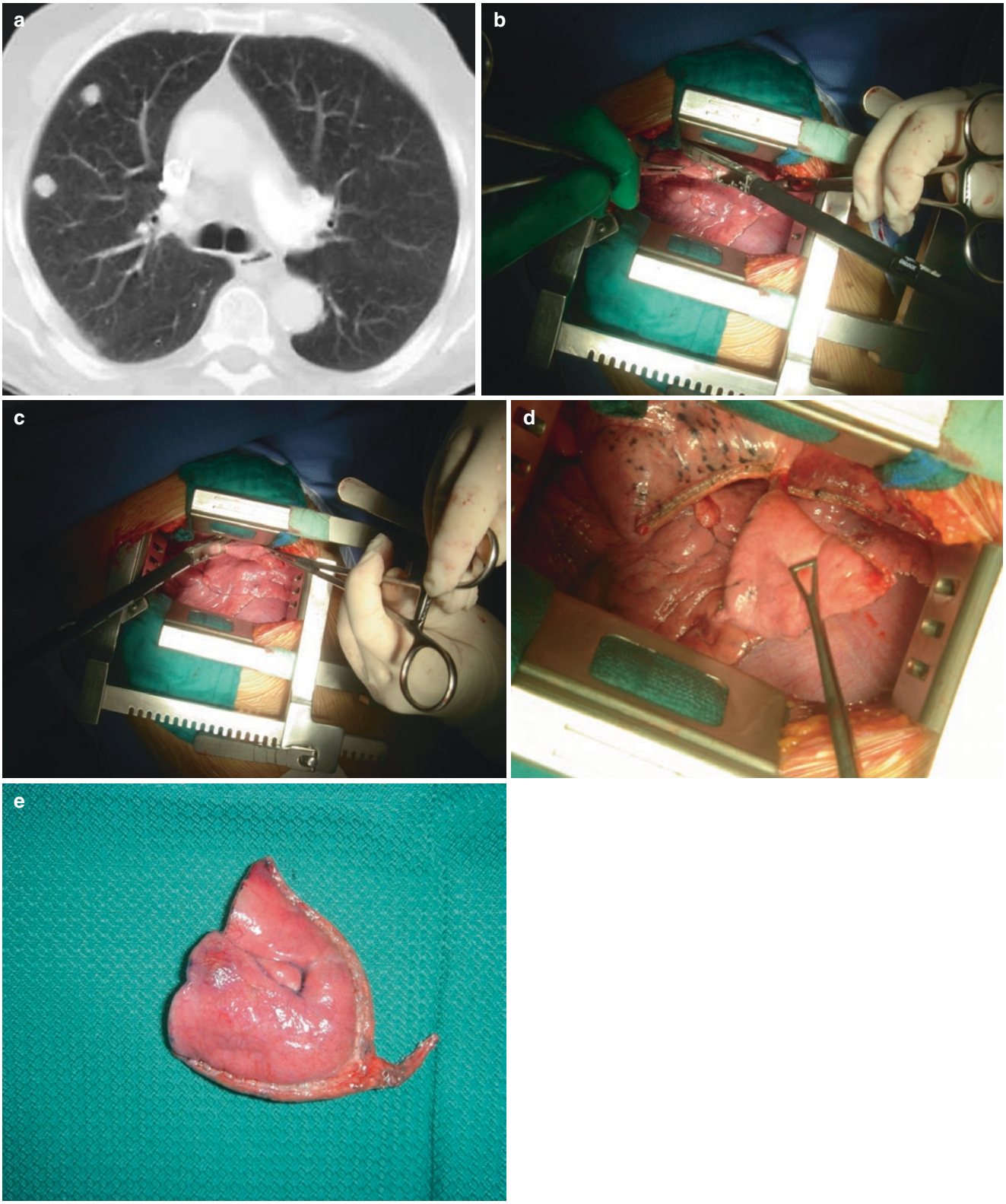


Fig. 31.1 (**a**) Monolateral lung metastases, amenable to surgical resection. (**b**) Identification and resection planning of a peripheral lung nodule. (**c**) Wedge resection using a surgical staple. (**d**) Result of lung resection. (e) Specimen with the evidence of a metastatic node

Pulmonary resection or metastasectomy offers an opportunity for long-term survival in highly selected patients with metastatic breast cancer, with case series demonstrating 5-year overall survival ranging from 30% to 80% and median survival duration ranging from 40 to 100 months [10, 19, 21, 27]. Based upon these observations, aggressive resection of isolated pulmonary metastases has become a widely accepted treatment for appropriately selected patients. The decision to proceed with pulmonary metastasectomy requires a multidisciplinary approach (a medical oncologist and thoracic surgeon). The goal is to offer surgery only to those patients who are most likely to benefit, to optimize the timing of surgical intervention. Patients who experience long-term survival after metastasectomy are those with solitary metastases and a disease-free interval greater than 36 months. In one report, the most important factor that influences survival is the positivity to HER-2; the 5-year survival moves from 12.1%, HER-2 negative, to 76%, HER-2 positive. Other prognostic factors may include size of metastases, unilateral disease, completeness of resection, and use of anatomic resection (as opposed to wedge resection) [10, 12].

31.2.10 Liver Metastases

Hepatic metastases occur in over half of patients with metastatic breast cancer, are commonly associated with disseminated disease, and result in a poorer prognosis than bone or soft tissue metastases.

Only 5–12% of patients have isolated liver involvement [31–33].

Only appropriately selected patients with breast cancer metastases may have an advantage from hepatic resection.

In a systematic review of 19 studies involving 535 patients who underwent hepatectomy for metastatic breast cancer, median overall survival was 40 months (range, 23–77 months) with a 5-year survival following resection of 40% (range, 21–80%) [34]. Postoperative mortality ranged from 0 to 6% and the complication rate ranged from 0 to 44%. Prognostic factors following hepatic resection were positive margins and hormone-refractory disease.

The ideal candidate has a solitary metastasis, no evidence of extrahepatic metastatic disease, normal liver function, a good performance status, and a long DFI [12, 14, 17].

An essential part of the diagnostic work-up in patients who are considered for hepatectomy is precise imaging of the liver (helical computed tomography scan or magnetic resonance imaging) to evaluate whether a complete resection can be achieved while retaining a sufficient volume of functional liver.

It is not clear whether multiple metastases are a negative prognostic feature for resection of liver metastases, as long as they can be completely resected [14, 15, 20].

Initial laparoscopic exploration may spare unresectable patients the morbidity of a laparotomy, since up to half of the patients considered for resection are discovered to have diffuse liver lesions or peritoneal dissemination at the time of laparotomy [35].

Hepatic resection is appropriate for highly selected patients, but alternative local therapies are being increasingly used to treat liver metastases.

Liver-directed therapy includes image-guided thermal ablation (radiofrequency ablation, microwave ablation, cryoablation, interstitial laser therapy), stereotactic body RT (SBRT), and intra-arterial therapies (selective internal RT and transhepatic arterial chemoembolization).

None of these methods has been directly compared to systemic chemotherapy in metastatic breast cancer, except in a recent paper addressing a cost-utility analysis of liver resection in breast cancer liver metastases. The authors compared postoperative conventional systemic therapy versus conventional therapy alone versus newer targeted therapy alone. The implications of using different chemotherapeutic regimens based on estrogen receptor and human epidermal growth factor receptor 2 status were also assessed. Liver resection in patients with breast cancer liver metastasis proved to be cost-effective when compared with systemic therapy alone, particularly in estrogen receptor-positive tumors or when newer agents were used [36].

31.2.10.1 Interventional Oncology

- Image-guided thermal ablation:

This category of local treatment includes modalities having the common aim of targeted tissue destruction by means of different forms of energy.

Those treatments can be used alone percutaneously, based on imaging guidance, or in combination with resection [31–34].

When radiofrequency energy is applied, an oscillating electrical current flows through the body inducing ionic agitation in tissues around the interstitial electrode. Resistive heating is produced in the areas closest to the interstitial probe [37] (Figs. 31.2, 31.3, 31.4).

Microwave (MW) ablation is based on electromagnetic energy, with frequencies greater than or equal to 900 MHz, which induces a vigorous movement of water molecules. This movement produces heat and thus tissue destruction and cell death via coagulative necrosis [38–41].

Cryoablation is based on alternating temperature decrease (−40 °C) and thawing. Rapid expansion of pressured gas within the probe creates a very low temperature and the formation of an iceball on the probe tip. Thawing of the iceball was achieved by insufflations of high-pressure gas. This system induces cell death by osmotic shock [42].

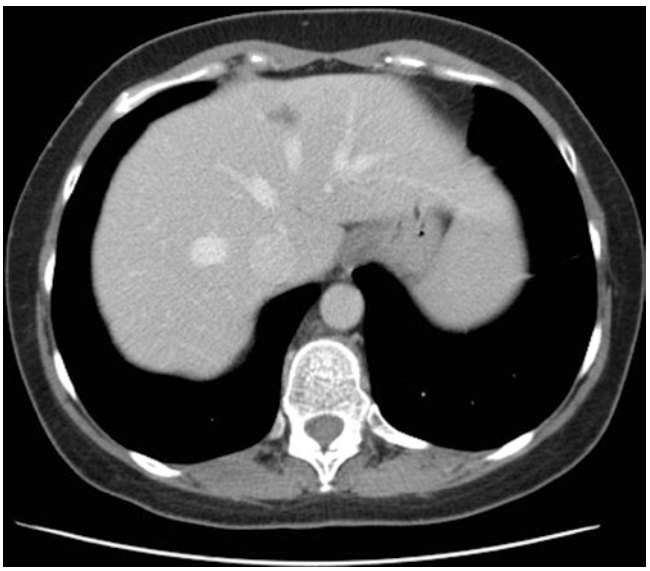


Fig. 31.2 CT scan portal venous phase: single metastatic lesion in segment II (pre)

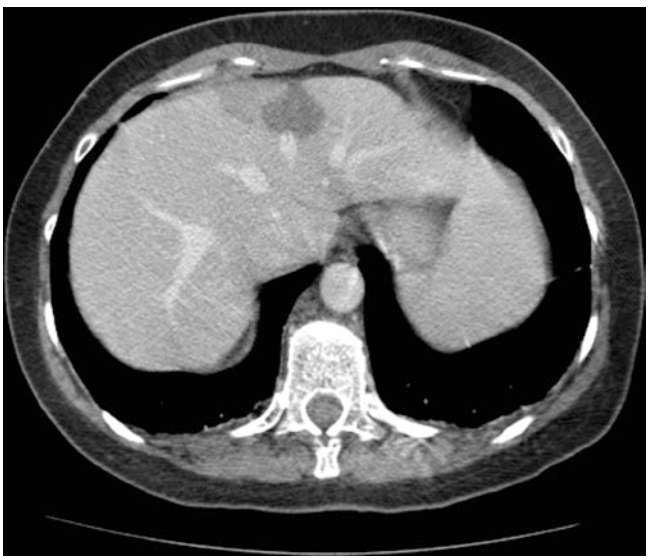


Fig. 31.3 CT scan the day after treatment showing complete treatment: hypodense ablation area without suspicious contrast enhancement

Interstitial laser therapy (ILT) causes local tumor destruction by the application of laser light, delivered through quartz diffusing laser fibers which can be percutaneously placed within tumors [43–46].

- Intra-arterial therapies:

The rationale behind transarterial treatment is that liver tumors receive blood flow almost entirely from the artery, while normal liver tissue is supplied both by the portal blood flow and by arterial blood flow. Selective intravascular delivery of agents into arterial tumor-feeding vessels has the goal of inducing lethal damage to the pathologic tissue while reducing collateral injury to healthy liver tissue [47, 48].

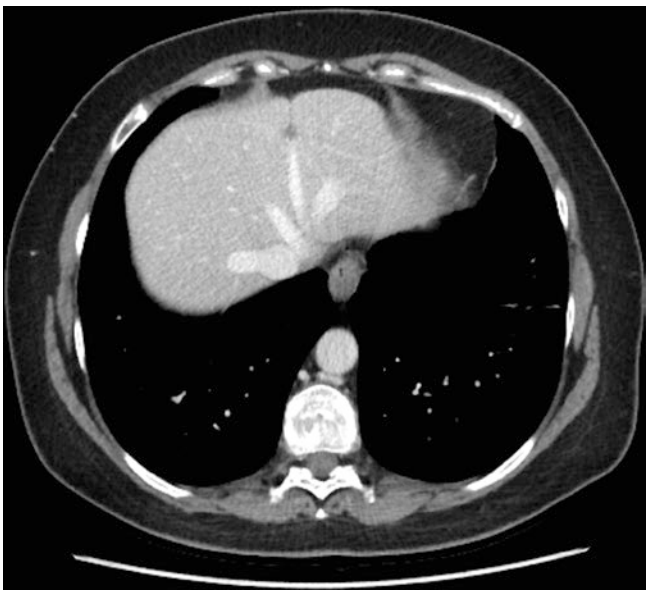


Fig. 31.4 CT scan 5 years after treatment showing shrinkage of ablation area. Patient is still disease-free

Different materials have been used in past decades in the treatment of primary and secondary liver cancers.

Transarterial chemoembolization (TACE) is based on different drug carriers (Gelfoam, microparticles) that have a dual aim: to reach higher intrahepatic drug concentration than that in systemic therapy and occlude arterial vessel to induce tissue ischemia.

Selective internal RT (SIRT) is a procedure in which glass or resin microspheres incorporating the radioactive isotope 90Y are directly infused into the hepatic arteries feeding the tumor. This will allow the delivery of doses of ionizing radiation above 120 Gy to the tumor compartment without causing intolerable toxicity to the normal tissue [49].

- Stereotactic body radiation therapy (SBRT or fractionated radiosurgery) is a technique that delivers external beam radiation to the tumor.

31.2.11 Brain Metastases

Breast cancer is the second most common cancer associated with brain metastases in the United States. In a subset of women, progression in the central nervous system (CNS) has become the major life-limiting problem. The risk of central nervous system (CNS) relapse among patients with breast cancer varies significantly by disease stage. Among women presenting with early-stage breast cancer, less than 3% will

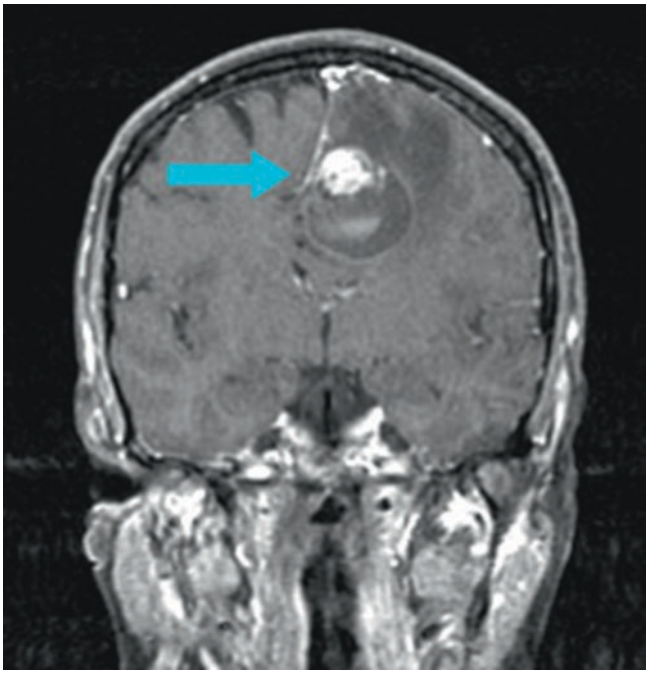


Fig. 31.5 Isolated brain metastasis

go on to develop brain metastases [1, 2]. In contrast, symptomatic brain metastases are diagnosed in 10–16% of patients with metastatic breast cancer [1, 3] (Fig. 31.5). Factors associated with an increased likelihood of CNS relapse include age under 40 years, pulmonary metastases, and African-American ancestry [1, 3–7].

In addition, the breast cancer subtype appears to be associated with the incidence of brain metastases [2, 6, 8–19]. In a cohort study of 1434 women treated with breast-conserving therapy plus systemic chemotherapy, brain metastases developed in 36 (2.5%).

For patients with a favorable prognosis (e.g., Karnofsky performance score [KPS] 70 or higher, age <65 years, controlled primary tumor, and controlled or absent extracranial metastases), aggressive treatment is indicated. For women with brain metastases from human epidermal growth factor receptor 2 (HER-2)-positive disease, the administration of systemic therapy may delay the use of whole-brain radiation therapy (RT) and the associated risk of neurologic toxicity.

Following initial treatment for brain metastases due to breast cancer, both surgery and stereotactic radiosurgery can be used to retreat patients who develop recurrent, symptomatic central nervous system (CNS) disease and have stable extracranial disease following their initial treatment for brain metastases. Careful selection of patients is critical in this setting. As with the initial evaluation, the absence of active systemic disease and a Karnofsky performance score of at least 70 are important. Factors indicating a probable poor outcome include a short time to recurrence and age less than 40 years.

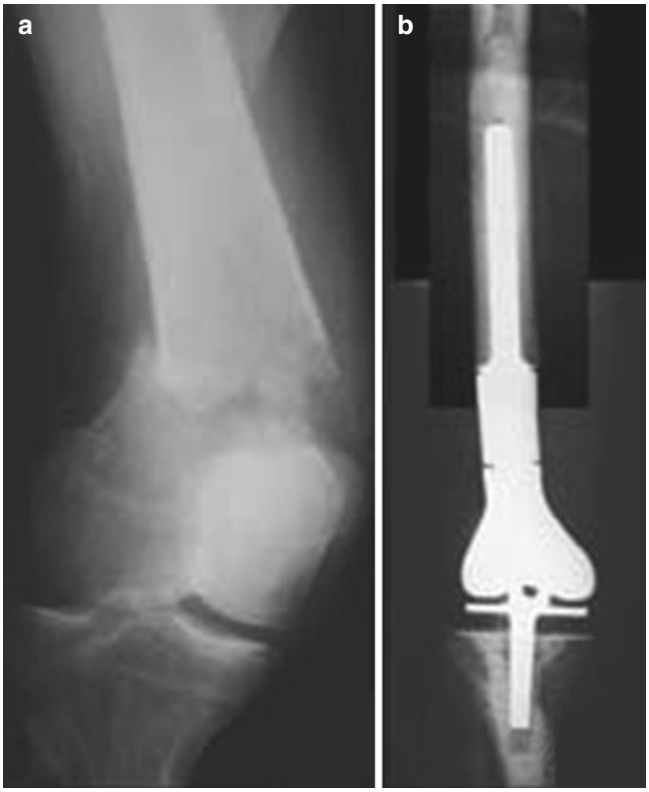


Fig. 31.6 Pathologic fracture through a metastatic lesion of the distal femur (**a**). Resection of the metastatic lesion and distal femoral replacement (**b**)

31.2.12 Bone Metastases

The bone is the most common site of metastatic involvement in breast cancer and can be associated with significant morbidity and mortality.

Surgery, RT, and RFA can provide effective pain relief and prevent fracture (Figs. 31.6 and 31.7). Surgery and RT are also used for the palliative treatment of epidural spinal cord or nerve compression.

Bisphosphonates and other osteoclast inhibitors have been shown to reduce the morbidity of metastatic bone disease, in particular skeletal-related events (SREs), which include fracture, need for surgery or radiation to the bone, spinal cord compression, and hypercalcemia of malignancy.

Bone-confined metastatic breast cancer is usually characterized by an indolent course and good response to systemic therapy [50–52].

There is a limited role for resection as a curative option for the majority of bone metastases, except for selected patients with isolated spine or sternal involvement [53–57].

Sternal metastases may remain solitary for a long time, possibly because there is no communication with the paravertebral venous plexus through which cancer cells can spread to other bones [53, 55].

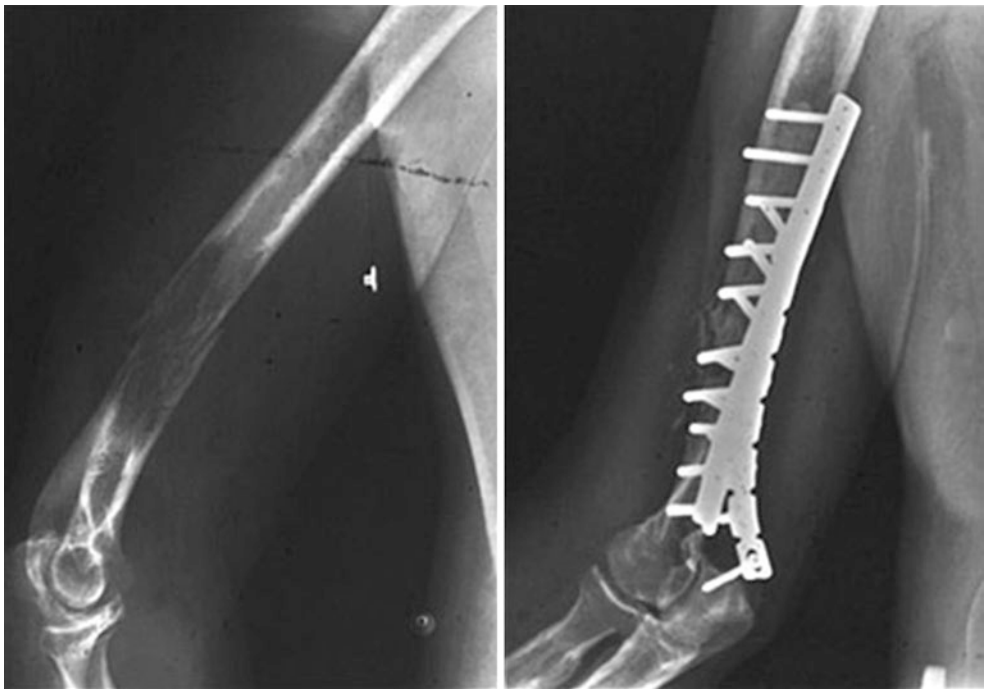


Fig. 31.7 Reconstruction of a pathologic fracture of the humerus with plates and screws

In other cases, isolated sternal involvement represents locoregional recurrence (i.e., direct extension from an internal mammary nodal recurrence) rather than true metastatic disease. Surgical resection of breast cancer confined to the sternum may improve quality of life and prolong survival.

When spine metastases become symptomatic causing severe pain, neurological deficit, and biomechanical instability, this may require surgical resection. The ideal approach is multidisciplinary and includes medical treatment (mostly for symptom control) radiotherapy, stereotactic radiosurgery, and surgery. The aim of surgery is to preserve or restore a neurological function in tumors that progress despite undergoing maximal radiation dosages and medical intractable pain. The treatment improves patient's quality of life; the indication for surgery should take into consideration anatomical location and the extension of disease [56, 58].

31.2.13 Abdominal and Pelvic Metastases

Limited data suggest that ovarian breast cancer metastases can appear many years following the initial diagnosis of breast cancer and tend to be hormone receptor-positive [59–62].

Surgical evaluation of an adnexal mass may be required to discriminate metastatic breast cancer from a primary ovarian cancer.

31.3 Summary and Recommendations

Local therapy may offer therapeutic benefits and the potential for long-term survival for highly selected patients with metastatic breast cancer.

Although most evidence exists in support of surgical resection, alternative approaches, such as radiofrequency ablation and stereotactic body radiation therapy, are gaining popularity.

Patient selection is crucial when considering local therapy. The best candidates are those with solitary metastases in a single metastatic site and a long disease-free interval.

Completeness of resection is a key factor when considering surgery.

Perhaps the main indication for resection of a new, isolated pulmonary, hepatic, or abdominopelvic lesion in a patient with a prior history of breast cancer is diagnostic, since some patients may have a new primary malignancy or a change in tumor marker status.

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Part VI

Plastic and Reconstructive Surgery

Oncoplastic Surgery

32

Cicero Urban and Mario Rietjens

32.1 Introduction

Oncoplastic surgery (OP) represents an important evolution in breast cancer treatment. As a surgical method and a technical refinement, it allows better aesthetic and oncologic outcomes. In this way, as a consequence of a more individualized approach, it can positively influence psychological aspects of patients and broadens indications for breast-conserving treatment (BCT). Therefore, the emphasis of this new phase in breast cancer surgery is on immediate reconstruction and contralateral surgery for symmetry whenever necessary, synergistically combining oncologic and aesthetic concepts by the surgical team or by a single surgeon [1–18].

Around 20–30% of BCT has unsatisfactory aesthetic outcomes and 10–40% of reoperations due to compromised margins [2–20]. In addition, defects after BCT tend to accentuate with radiotherapy, increasing asymmetry, which usually require flaps or lipofilling to correct them in the future. But, unfortunately, aesthetic outcomes after delayed partial breast reconstructions are many times unsatisfactory [18–30]. Then OP is the way to reduce the conflict in BCT of performing resections with free margins even in large and multifocal tumors and does not remove so much breast tissue, which could result in major deformities and asymmetry between the breasts [3–8].

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So, every effort should be made to identify better candidates to this surgical approach. If local-regional control represents the main target for oncologic surgeries, aesthetic outcomes and quality of life are also basic principles in BCT, from the very beginning [31, 32]. Then, in this chapter, it will be discussed OP history and evolution, and the indications and limits of Class I and Class II techniques in BCT.

32.2 History and Evolution

Historically it is difficult to precisely define the first time that a mammoplasty technique was used in BCT with the aim of reducing deformities and asymmetries. There were a number of nonacademic surgeons, in different countries, who were doing sporadically this kind of surgery, even before its appearance in the literature. One of its earlier applications was in the 1980s in France by Jean-Yves Petit (at that time at Institut Gustave Roussy), Jean-Yves Bobin (Centre Léon-Bérard), and Michel Abbes (Centre Lacassagne). Some years later, the OP concept was then originally coined by Werner Audrescht in German and posteriorly had a major diffusion after the publication of the classic paper from Krishna Clough and colleagues in 2003 (Fig. 32.1) [3, 9]. In Brazil, some breast surgeons like Antonio Figueira, Angelo Matthes, and Jorge Biazús were doing OP since the 1980s too. And, despite the lack of randomized trials, current evidence suggests at least equivalent oncologic outcomes, reduced re-excision rates, and superior aesthetic results when compared to lumpectomies (Table 32.1). Therefore, although it remains not a consensus, the original OP concept as "tumor-specific immediate reconstruction" [1] is not limited to BCT. Skin-sparing and nipple-sparing mastectomy techniques have incorporated OP principles doing a well-conducted oncologic resection followed by immediate breast reconstruction and contralateral symmetry in the same surgery.

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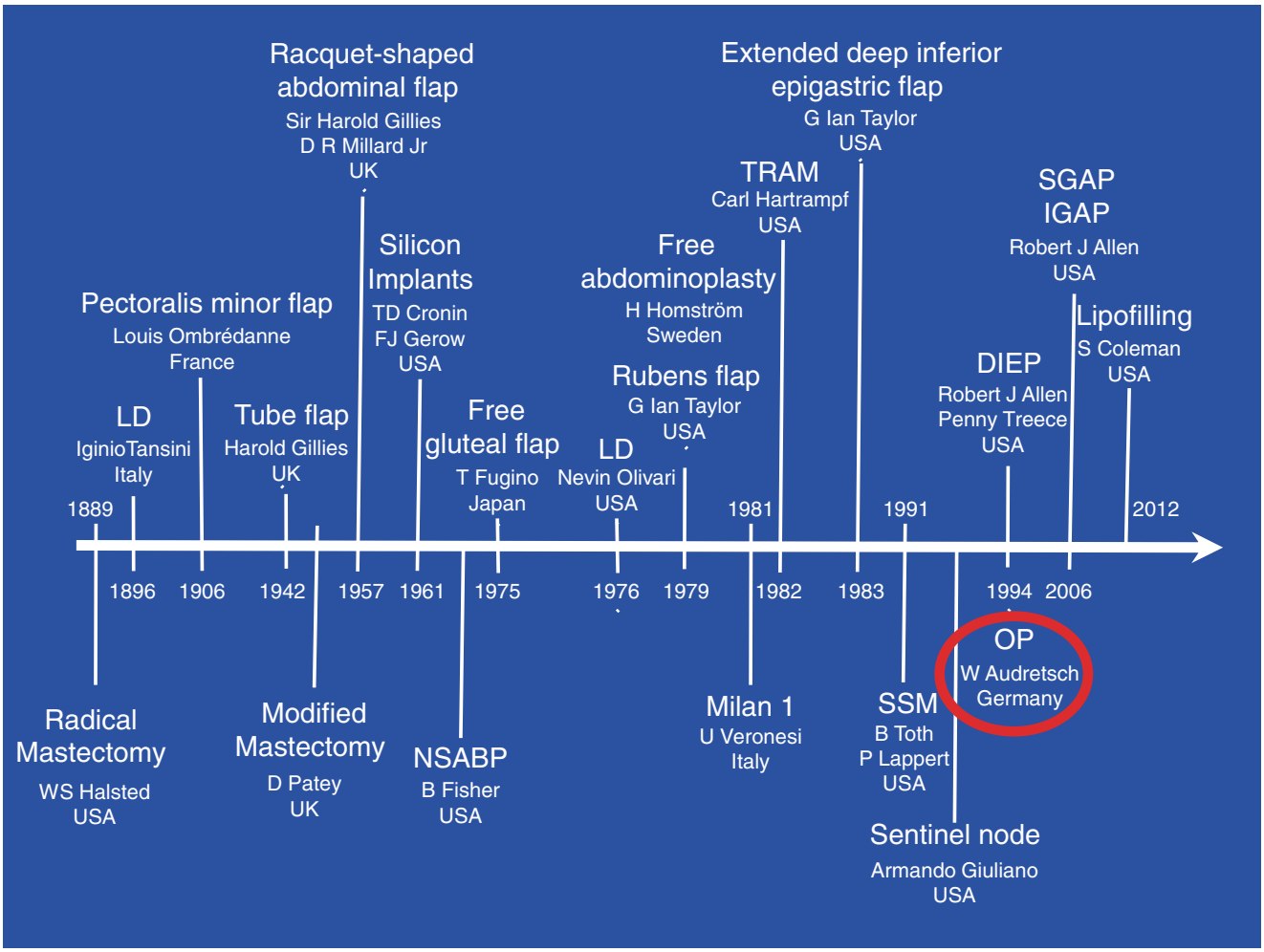

Fig. 32.1 Historical milestones in breast cancer surgery and in breast reconstruction

Table 32.1 Oncologic and aesthetic results in oncoplastic surgery

Author	Year	*n*	Tumor size (cm)	FW (months)	Margin involvement (%)	LR (%)	Cosmetic failure
Clough et al.	2003	101	3.2	46	10.9	6.9	12
Losken et al.	2007	63	NR	40	NR	2	5
Rietjens et al.	2007	148	3.2	74	5	3	8.9
Munhoz et al.	2008	209	NR	31	5.7	5.7	7.7
Fitoussi et al.	2010	540	2.9	49	18.9	6.8	9.7
Urban et al.	2012	109	1.5	NR	7.5	NR	NR

NR non-related

32.3 Oncologic and Aesthetic Outcomes

Although there are no randomized trials comparing OP with standard BCT and many reported series are retrospective and noncontrolled ones, the current data is enough to incorporate OP in current BCT (Fig. 32.2) [1–29]. In addition, OP follows the same BCT oncologic principles (Fig. 32.3). Haloua [26], in their review of 11 prospective oncoplastic studies, found 7–22% positive margin rate in OP, compared to the 20–40% in standard BCT. This difference resulted in lower re-excision rate. Santos, in Brazil, did a comparison between quality of life and aesthetic outcomes in OP and lumpectomy and found that excellent aesthetic outcomes are more frequent in OP [4]. A recent meta-analysis by Losken [27] also demonstrated larger resection volumes, increased satisfaction with aesthetics, and decreased rates of positive margins, re-excisions, and local recurrences for OP. No significant delay in adjuvant chemotherapy and radiotherapy was related

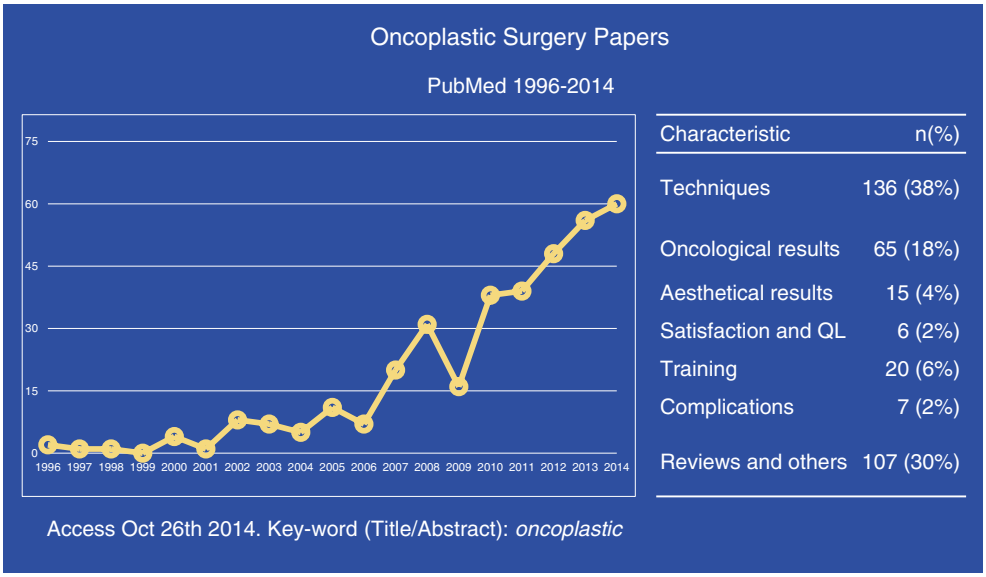

Fig. 32.2 Evolution and characteristics of oncoplastic surgery papers

despite the increased complexity of these surgeries [6–8, 26, 27]. Long-term survival has been equivalent to BCT series [26, 27]. A valid concern over the OP approach is the reliability of clips placed for the boost, although advances in intraoperative radiation therapy may make this less of an issue. Tissue rearrangement during oncoplasty might result in a larger, less exact boost during external beam radiation therapy, possibly resulting in a poorer aesthetic outcome and decreased local control of disease.

32.4 Patient Selection

Classically, the most frequent deformities after BCT are deficiency of glandular tissue and overlying skin retractions resulting from wide resections and late side effects after radiotherapy, deformity and/or retraction of the nipple and areola complex (NAC), and reduction of mammary ptosis and asymmetry of the inframammary crease as a consequence of fibrosis and retraction after radiotherapy. All these deformities are expected to be more evident when the relation tumor/breast is unfavorable, and they are also related to the tumor location and its proximity with the NAC and skin, and the boost. So, the most adequate technique for each patient should be determined according to the anticipation of the size and position of the future defect, tumor proximity with the skin and NAC, and clinical conditions of the patient [1–17].

Class II OP is more complex and time consuming than classic lumpectomy. Thus the selection of patients from oncological, aesthetical, and psychological point of view is critical. All attempts should be made to minimize the risk of positive margins, which are difficult and sometimes impossible to reassess in a second surgery [30], and to reduce and prevent complications that may delay adjuvant treatments. Therefore, there are some established indications for OP in BCT; the main ones are for patients with more than 20% of volume of mammary resection, and especially in the case of macromastia, where results from skin-sparing or nipple-sparing mastectomy are usually unsatisfactory, and OP approach may also favor radiotherapy planning.

Current indications and limits of Class II OP in BCT are in Table 32.2.

32.5 Preoperative Planning

Although the only significant element referred to as an aesthetical risk for BCT in Cochrane evaluation was the volume of mammary resection over 20%, in clinical practice there are many other risk factors that should be observed [8]. The choice of OP technique depends on tumor location and size, multifocality, multicentricity, bilaterality, breast size, ptosis, shape and symmetry, previous mammoplasties, and previous radiotherapy [8].

In some circumstances, some associated clinical conditions may also influence the choice of the most appropriate technique. Diabetic patients, obese patients, tobacco addicts, those with collagen diseases, and those above 70 years old are subject to risks concerning unsatisfactory aesthetic results, and skin healing complications are higher. Major resections and wide NAC dislocations may bring additional risks of fat necrosis and of partial or total NAC losses [8].

The ideal location for a tumor is within the mammoplasty area. When the tumor is close to the skin and out of this area, the OP procedure may be more complex, and it may require combined techniques, whose results are not

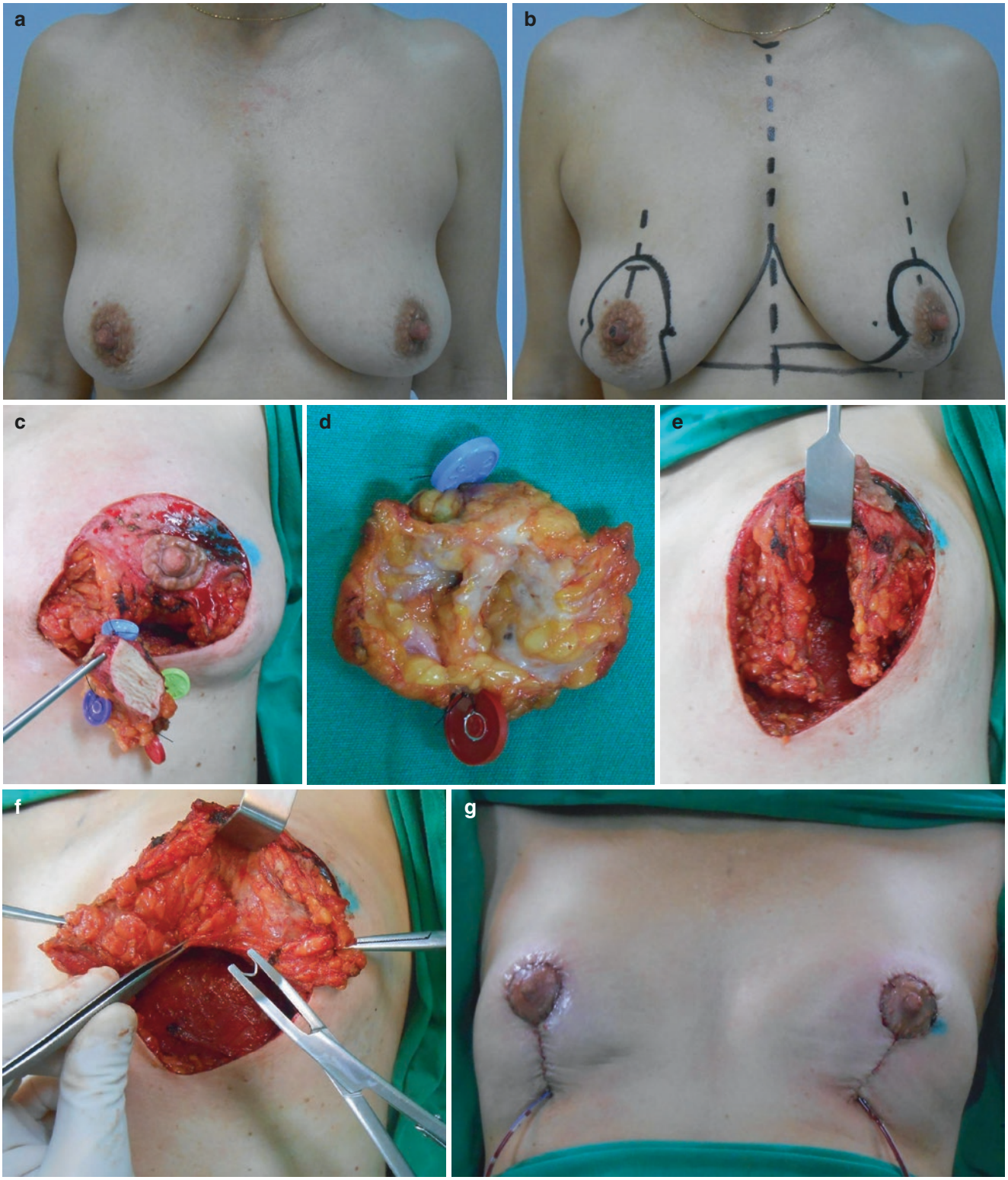

Fig. 32.3 Oncoplastic surgery step by step: (**a**) preoperatory view of a 50-year-old patient with T1cN0 breast cancer in the inferior quadrant of the left breast; (**b**) preoperatory draws for the surgical planning; (**c**) resection of the tumor (with skin over it); (**d**) tumor in the center and demarcation of the margins to guide the pathology; (**e**) the two pillars for partial breast reconstruction; (**f**) surgical clips for the boost; (**g**) final result with contralateral mammoplasty for symmetry

Table 32.2 Indications and relative contraindications for Class II oncoplastic surgery in breast-conserving surgery

Indications
• Resections over 20% of breast volume
• Macromastia
• Severe ptosis and asymmetry
• Need for large skin resections inside mammoplasty area
• Central, medial, and inferior tumors
• Previous plastic surgeries in the breast
Relative contraindications
• Extensive tumors located in medial regions
• Low-volume breasts, particularly those without ptosis
• Previously irradiated breasts
• Skin resections beyond mammoplasties zone in small-/medium-size breasts
• Tobacco addiction and uncontrolled diabetes
• Exaggerated patient's expectations with aesthetic outcomes

always satisfactory. In such cases skin-sparing or nipple-sparing mastectomy should be considered as an option. Flaps as the one from the *latissimus dorsi*, which has a different color and texture from the breast, usually bring unsatisfactory results and therefore should be considered as an exception [8].

High-volume breasts, with severe ptosis, allow for surgeries with wider margins and usually bring more satisfactory results. Patients with macromastias present a formal indication for OP due to better radiotherapy planning in a smaller and round breast. In cases of previous breast augmentation plastic surgery, it is necessary to take into consideration that the breast volume is not the real one, and consequently some considerable deformities may result. The biggest problem concerning OP is dealing with young patients, with conic breast, without mammary ptosis, and with low or medium volume. In such cases, according to the location or tumor size, local flaps offer a little chance of good results, so skin-sparing or nipple-sparing mastectomy with immediate reconstruction may be the best choice [8].

Basically, the flowchart for OP planning which we use in our practice takes into account both breast and tumor characteristics, and it is presented in Fig. 32.4.

32.6 Class I Techniques

32.6.1 Glandular Flaps

Class I techniques consists of moving glandular flaps around the defect caused by lumpectomies, in an attempt to cover it completely. It is preferentially indicated for premenopausal patients, when the glandular component of the breast is higher, therefore reducing the risks of liponecrosis in the postoperative period. This technique is also indicated in cases of tumors located in the upper quadrants, where the mammary gland is less thick; and even if there is a small filling defect, such a defect is not so visible. The opposite effect happens in the lower quadrants, where the mammary gland thickness is more evident and where adapted techniques are necessary. Glandular reshaping in lower portions of the breast is possible for small tumors and in a vertical or oblique way.

32.6.2 Central Quadrant Techniques

This represented a great innovation in early days of BCT, as up to some years ago having a retroareolar neoplasia was synonymous of mastectomy [17]. Immediate breast reconstruction techniques for central quadrant resections may vary according to breast volume, level of ptosis, and shape of ptosis (either vertical or lateral). Considering breast without ptosis or with slight ptosis, it is possible to use the glandular suture in tobacco pouch. Two or three layers of glandular suture in tobacco pouch allow for obtaining the central projection of the mammary cone, and the intradermal suture also in tobacco pouch would produce a residual scar within the area where the future NAC would be reconstructed, therefore causing the scar to disappear almost completely. The disadvantage of this technique is that there might be delay in the healing process. In medium- or large-size breasts with some degree of ptosis, it is possible to use Grisotti's technique, which is derived from the reduction mammoplasty techniques, with the rotation of infero-lateral glandular pedicle, preserving a coetaneous island that replaces NAC. This might be the first OP technique described in the literature, as it was a direct adaptation of plastic surgery technique to BCT [17]. For some large breasts, it is possible to do a reduction mammoplasty like Pitanguy's, but resecting the NAC.

32.7 Class II Techniques

The great diversity of mammoplasty techniques used in aesthetic surgery supports an increase in the indications of BCT. In most cases, reductive mammoplasties based on different pedicles can be transported to BCT. The level of mammary ptosis, differences of volume and shape detected in the preoperative stage, level of mammary liposubstitution, height, shape and size of the NAC, and mainly the size and location of the tumor are the most important factors to consider when choosing the technique of mammoplasty to be applied in BCT.

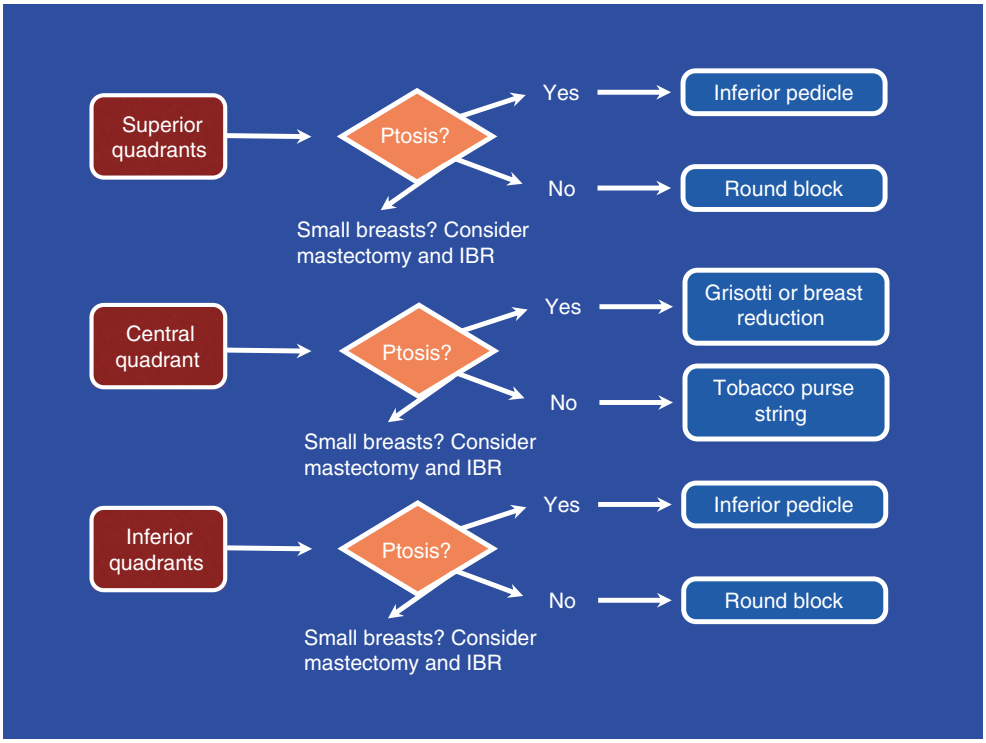

Fig. 32.4 Decision flowchart for Class II oncoplastic surgery in breast-conserving surgery

32.7.1 Periareolar Techniques

Class II techniques are inspired in reductive mammoplasty techniques proposed by Sampaio-Goes [33] and Benelli [34], in which a major glandular coetaneous undermining procedure for remodeling through a periareolar scar is performed. It is indicated for cases of non-ptotic (or with discrete ptosis) small- or medium-size breast. The great advantage of these techniques is that it allows lumpectomies in any part of the breast, except for the central quadrant.

32.7.2 Superior Pedicle Techniques

These techniques are based on superior vascular pedicles as those proposed by Pitanguy [35] and Lejour [36] in aesthetic surgery. They may be useful in cases of tumors situated in the lower quadrants and are appropriate for large and ptotic breasts or breasts medium size with some degree of ptosis. The decision whether perform only a vertical scar or an inverted "T" scar will depend on the level of hypertrophy and the level of ptosis. Considering smaller breasts and those with less ptosis, it is possible to perform only a vertical scar, and considering cases of larger breasts with a major ptosis, an inverted "T" scar will avoid the cutaneous excess. The format of the scar as vertical or inverted "T" can be central (more frequent), medial, or lateral, according to the location of the tumor and the need for skin removal.

32.7.3 Inferior Pedicle Techniques

These techniques are based on inferior-posterior vascular pedicles, as described by Ribeiro and Robbins, and they may be applied in cases of tumors situated in the upper quadrants of the breast [37, 38]. The preoperative drawing can be made in the same way and using measurements of Pitanguy's and Lejour's techniques, with a periareolar scar and inverted "T" or vertical/oblique inferior line. This is one of the most useful techniques, as there are many tumors in superior quadrants.

Conclusions

Surgeons play an influential role in the care of the breast cancer patient. OP allows for an oncologic-aesthetic-functional individualized surgical approach. Such an advance means a new philosophy in breast cancer surgery. It also brings new challenges for mentoring and training new generations of surgeons and opens new perspectives of research related to aesthetic results, quality of life, and local control of disease, as well as optimization of operative timing and reduction of both complications and costs. Finally, OP expertise is resulting in a higher standard of care for breast cancer patients.

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Delayed Breast Reconstruction

33

Cicero Urban and Flavia Kuroda

33.1 Introduction

Delayed breast reconstruction is thought to be the first technique for restoring the physical integrity after mastectomy. Until some decades ago, breast reconstruction could not be performed until 2 or even 5 years after conclusion of oncologic treatment [1, 2]. Today, immediate breast reconstruction can be indicated for most breast cancer patients, but unfortunately the majority of them remain without their breasts. And there are different well-documented reasons for that, such as disparities related to race, sociodemographic factors, and financial and some cultural barriers. Then, delayed breast reconstruction is an option for many patients [3, 4].

Implants and autologous reconstructions are the most important options. Indications for them depend on patient's anatomy, previous radiotherapy, or patient's preferences. Both magnitude of the procedure in terms of invasiveness and morbidity in each individual case are important points to consider. Implant-based breast reconstruction is notable for its surgical simplicity, applicability, and faster recovery time, but it is not allowed in all cases [5]. Despite of that, there are some limitations for such an approach, like previous radiotherapy or Halsted's mastectomy. It is also important to take into account patient's expectations in order to better individualize the decisions.

So, the aim of this chapter was to cover the indications, preoperative evaluation, operative techniques, and complications related to delayed breast reconstruction.

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33.2 Indications and Selection of Patients

33.2.1 Timing of Reconstruction

Delayed breast reconstruction can happen at any time, given that the wound has healed and adjuvant therapy has been already completed. But postradiation acute skin lesions and hematologic effects of chemotherapy should be completely ceased [6]. At the *Hospital Nossa Senhora das Graças* Breast Unit in Curitiba (Brazil), the routine is waited at least 6 months after the conclusion of adjuvant radiotherapy and 30–40 days after the end of chemotherapy. Different from the immediate approach, the delayed one can be indicated even for patients who had impaired perfusion of skin flaps after mastectomy [7]. Therefore, it is useful to be clear for patients who suffer from some medical comorbidities such as active smoking, diabetes, obesity, or cardiopulmonary disease that these conditions might predispose to some additional risks.

Delayed breast reconstructions have some facilities regarding the immediate ones because adjuvant treatment is already concluded. Moreover, there are series demonstrating that delayed has fewer complications [8]. However, the technique might entail other surgeries in order to ameliorate aesthetics, thus prolonging the overall time of treatment for patients, because it provides less cosmetic quality than the immediate reconstruction [7]. Furthermore, delayed reconstruction has limited reconstructive options following radiation therapy.

33.2.2 Implant-Based or Autologous Techniques

Delayed breast reconstructions can be implant-based or autologous-flap-based ones. The first technique involves the use of silicone-filled or saline-filled implants and definitive or temporary expanders beneath the remaining mastectomy skin flaps and the pectoralis major muscle, whereas the autologous reconstructions use musculocutaneous flaps, which consist of a segment of vascularized muscle with the overlying skin

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and fat, which are perfused by perforating vessels from the underlying muscle. It can be with pedicle or free flaps, and sometimes it is also necessary the association of an implant for better volume and projection, as it is the case with latissimus dorsi flap. While for some patients the overall result is more pleasing with musculocutaneous flaps [3, 4, 7], there are some disadvantages, which include longer surgical length and prolonged postoperative recovery when compared to implant-based reconstructions. In Fig. 33.3 there is a nice example of this, in a patient with previous breast cancer and radiotherapy in the thoracic wall and neurofibromatosis. Moreover, with implants there is no donor-site morbidity, reduced operative time, and more rapid postoperative recovery when compared to autologous reconstructions [9, 10]. In addition, with the new generation of breast implants, particularly the anatomical ones, it is possible to achieve good aesthetic outcomes and high rates of patient's satisfaction [20].

33.2.3 Definitive Implants or Temporary Expanders

In patients who were not previously irradiated, the choice of the most appropriate technique requires some specific preoperatory clinical evaluations: skin and musculocutaneous conditions in the mastectomy flap, size and ptosis of the contralateral breast, and patient's expectations about her breast reconstruction. For instance, the complete absence of the pectoralis muscles due to Halsted's mastectomy is a contraindication to this approach [11, 12]. Using a definitive form-stable implant rather than a temporary expander is not frequent in delayed reconstructions. The ideal patient for this approach should have a non-tense cutaneous flaps, a good quality of her pectoralis major muscle, and a small contralateral breast.

The tissue expansion with a temporary expander before to change to a definitive form-stable implant is the most frequent indication for delayed breast reconstruction for non-irradiated patients—the two-stage techniques. The expander is used to distend the cutaneous flaps in order to facilitate the insertion of definitive form-stable implant in a second surgery. The choice of the temporary expander is in a similar way of the definitive ones—basis, height, and desired volume should be considered. Older patients, those with significant medical comorbidities, and women with minimal abdominal tissue in whom the autologous technique would be unsuitable also benefit from this technique. Besides, the expander/implant technique is to be indicated for those patients devoid of sufficient skin or preserved subcutaneous tissue in flaps resulting from mastectomy. This may occur when there is little elasticity of the cutaneous flaps from mastectomy or in the case of a contralateral breast presenting a rather large volume. In these situations, the two-stage implant reconstruction usually yields aesthetically superior outcomes.

There are some cases where two-stage approach is contraindicated, and they are basically the same ones as those for definitive implants, with even more emphasis on the risk of expanders after radiotherapy [13]. Many authors have realized that several postoperative complications can ensue when attempting to distend previously irradiated tissues [13–16], since the radiation decreases the tissue elastic distension capacity. In these cases, the most frequent complications are painful and difficult expansion with possible extrusion of the expansion device or periprosthetic capsule. Even though one achieves the final stage of expansion, the cutaneous coverage of the prosthesis becomes too thin and fragile to protect the definitive implant. Recently, the addition of lipofilling in breast reconstruction armamentarium is allowing to expand irradiated tissues in selected cases, but it is necessary to have more data in this specific approach.

A practical flowchart for decisions in delayed breast reconstruction is shown in Fig. 33.1.

33.3 Preoperative Evaluation

The aim of breast reconstruction is to obtain symmetry [17, 18]. For this reason, it is essential to carry out a preoperative plan that includes a detailed analysis of the healthy breast's characteristics in order to make the correct choice of the most suitable technique to reconstruct the other breast [19]. It is important to remember that the reconstructed breast, most of the times, will have low projection in the upper pole and no ptosis. With these characteristics in mind, the contralateral breast should be planned to have an intervention for symmetry in the same surgery or in a second one (after the change of the temporary expander for a definitive implant).

Clinical and radiologic preoperative evaluations are crucial in order to clarify the patient's risks for the surgery. Diabetes, hypertension, obesity, and tobacco-using patients have higher risks for bad aesthetic outcomes and for implant or expander's extrusions. It is also important that a detailed oncologic evaluation be performed, surveying the following topics of the past treatment: type, localization, and size of tumor; number of positive lymph nodes; type of surgical procedure performed; chemotherapy; radiotherapy; hormone therapy; follow-up period; and the most recently performed radiologic and blood exams. Furthermore, the evaluation of the contralateral breast is also mandatory in order to exclude bilateral neoplasm and should include mammographic and ultrasound exams. In high-risk patients with hereditary breast cancer syndromes such as BRCA 1/2 mutations, it is necessary to add breast MRI. These exams are important because contralateral breast surgery—a reduction mammoplasty, mastopexy, or augmentation mammoplasty—is frequently required to obtain a more pleasing symmetry.

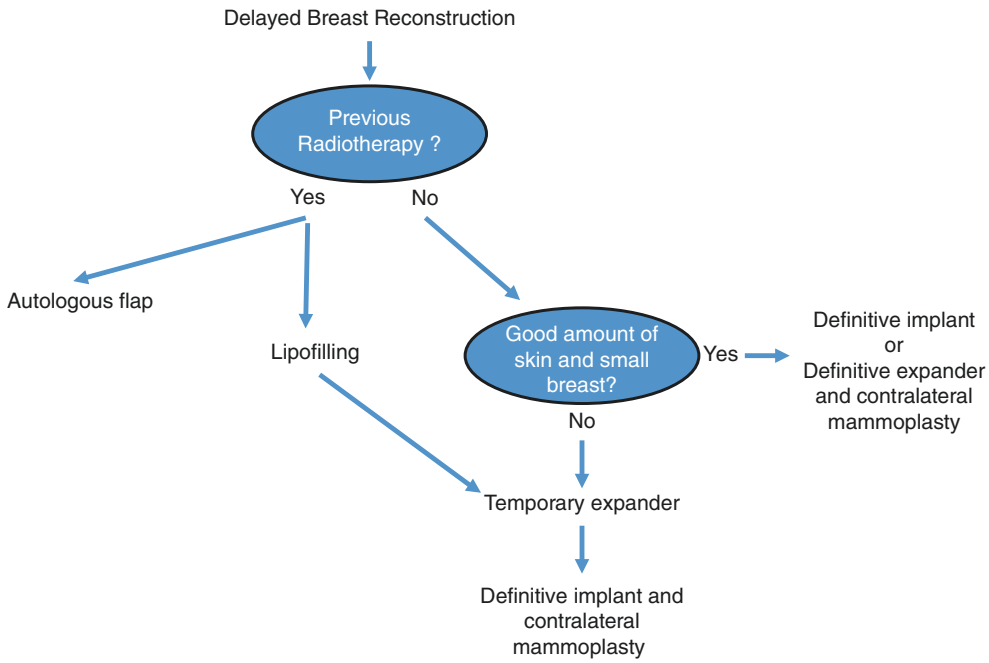


Fig. 33.1 A practical flowchart to guide decisions in delayed breast reconstruction

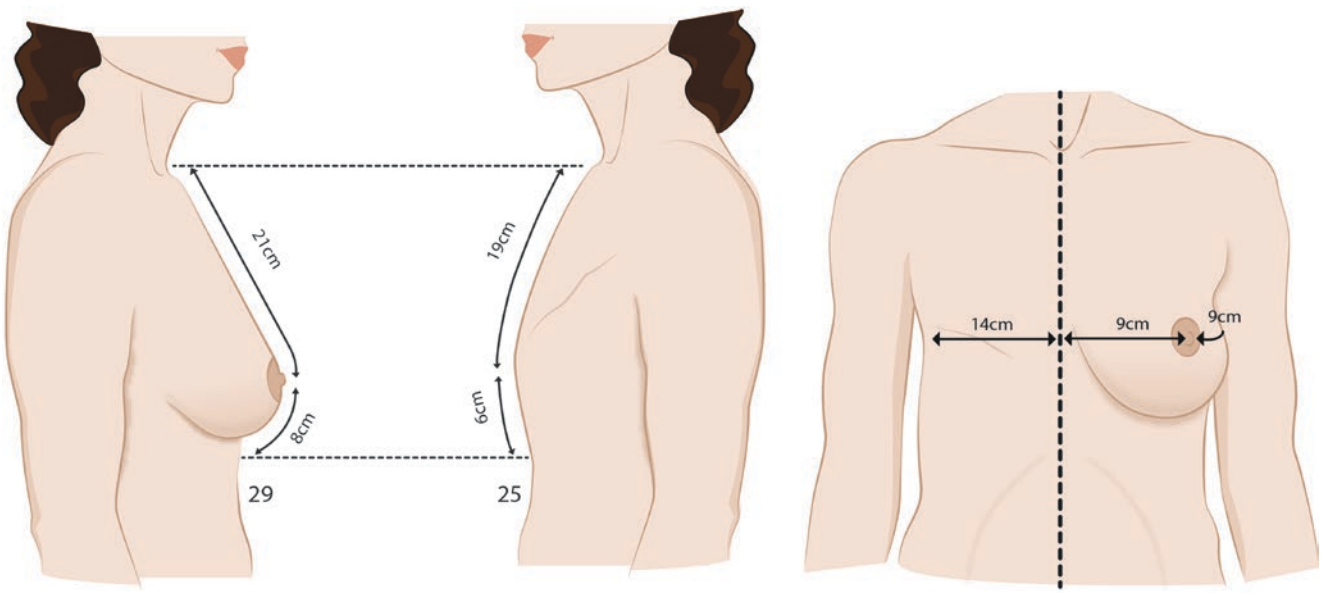


Fig. 33.2 Pre-operatory measurements for surgical planning and choice of the expander and implant

33.4 The Day Before the Operation

In the day before the operation, the whole procedure is explained to the patient again, and then the informed consent form is obtained. The patient is then placed standing, and photographs are taken in profile, in partial profile, and in forward-facing position. It is useful to make precise measurements of the contralateral breast in this occasion, such as base width, thickness of subcutaneous adipose tissue, and height as well as anterior projection (Fig. 33.2).

33.4.1 Choosing the Correct Expander and Implant

Concerning the decision as to which implant one should use, it is important to compare the contralateral breast with the future implant with regard to the parameters of base, height, and anterior projection. This is done during the preoperative period in order to choose two or even more models and sizes of implants that are most likely to be used during the surgical procedure. The final decision can be

made at the intraoperative stage, sometimes with the help of samples. Surgeons should pay attention to whether samples are prohibited in the country they work in. In Brazil there are some specific norms for that, and at the European Union, for instance, the re-sterilization of samples is strictly forbidden. Nevertheless, the non-sterilized implants can be thoroughly coated with a highly adherent and resistant sterile plastic envelope, therefore permitting their repeated usage. This technique for choosing the implants based on the aforementioned measures is precise and particularly useful in the cases in which it is necessary to use an expander/implant and, subsequently, perform a contralateral augmentation mammoplasty [20–22]. In cases of definitive implants with mastopexy or reduction mammoplasty in the contralateral breast, the decision as to the type and volume of the implant must also take in account the volume reduction, the change of shape, and the reduction of the breast base. These calculations can be based on augmentation mammoplasty papers [23, 24], which employ these methods to calculate the volume and shape of implants for aesthetic improvement, on samples, and in surgeon's personal experience (Figs. 33.3, 33.4, 33.5, and 33.6).

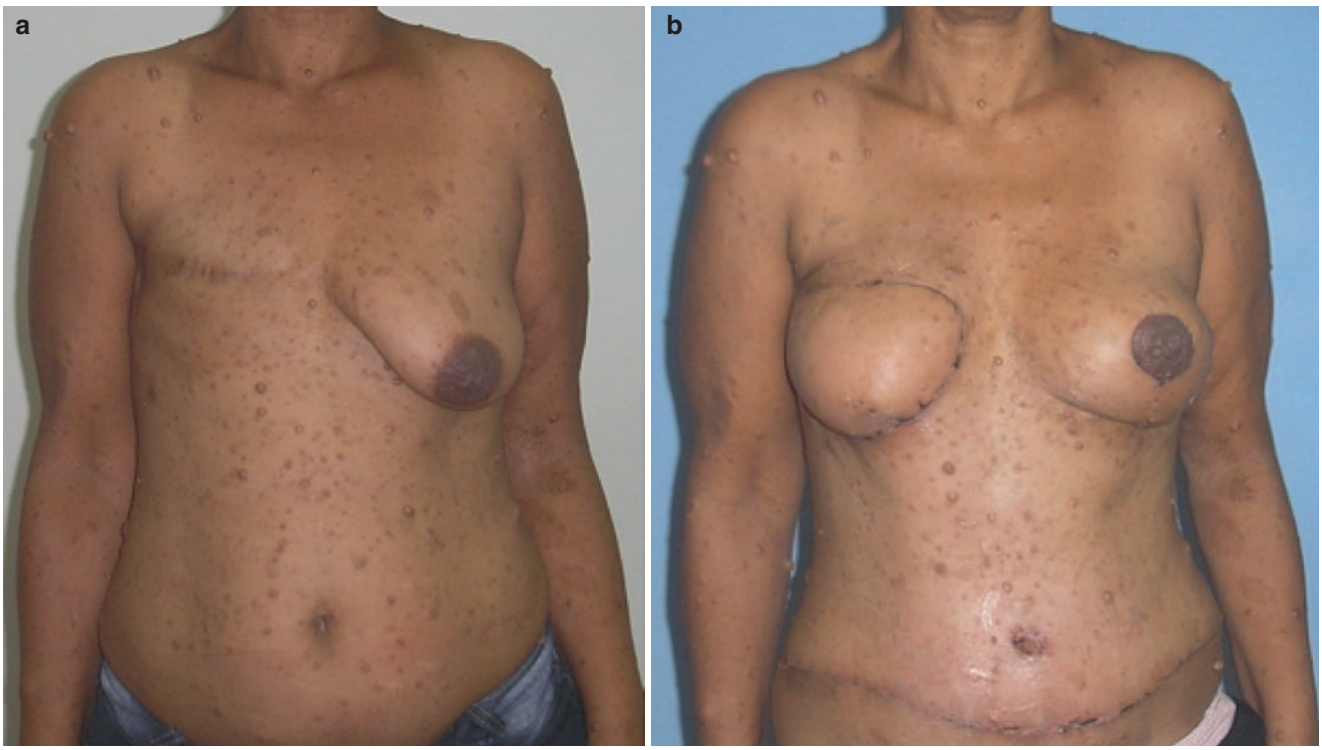


Fig. 33.3 Young patient with previous mastectomy, thoracic wall radiotherapy, and neurofibromatosis

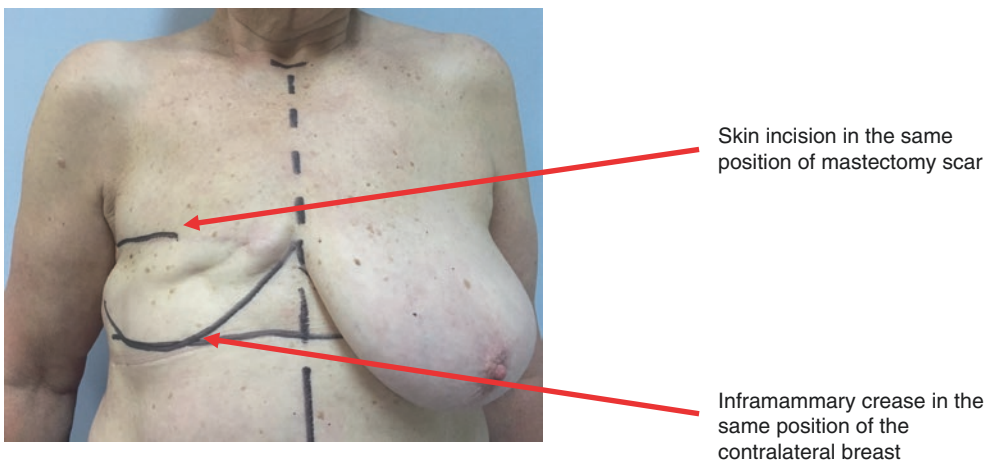
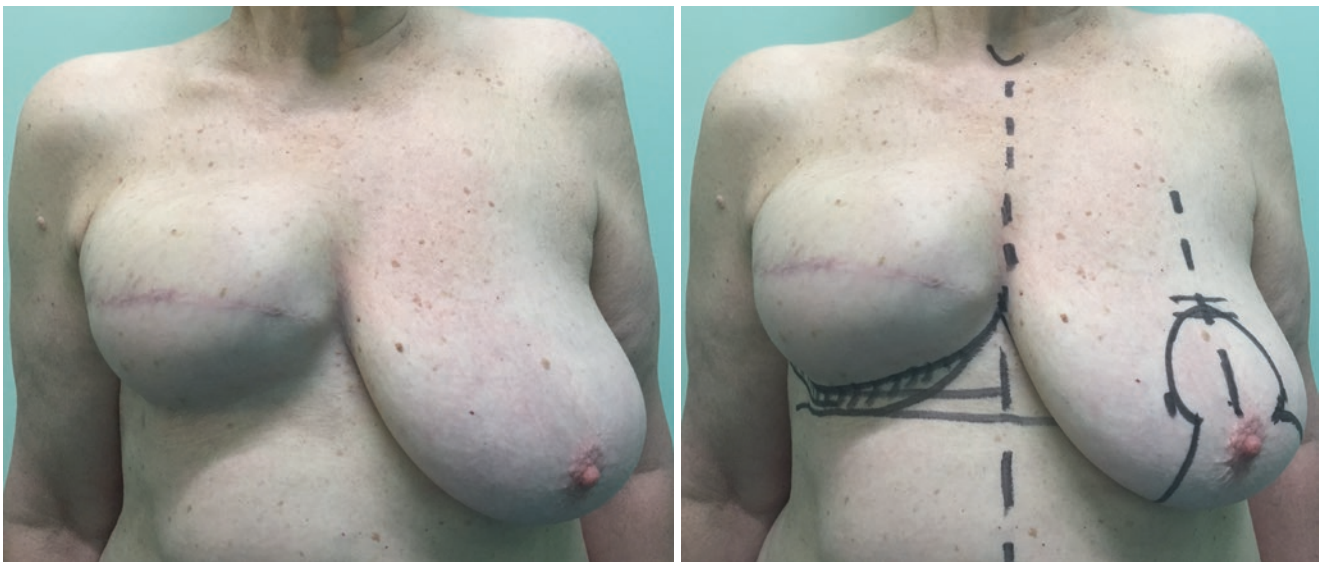


Fig. 33.4 Pre-operatory view with planning draws of a 70-year-old patient for delayed breast reconstruction with temporary expander



Final result after reconstruction with temporary expander

Pre-operatory draws for changing by definitive implant and contra-lateral breast reduction

Fig. 33.5 Pre-operatory view before changing temporary expander by definitive implant and contralateral breast reduction

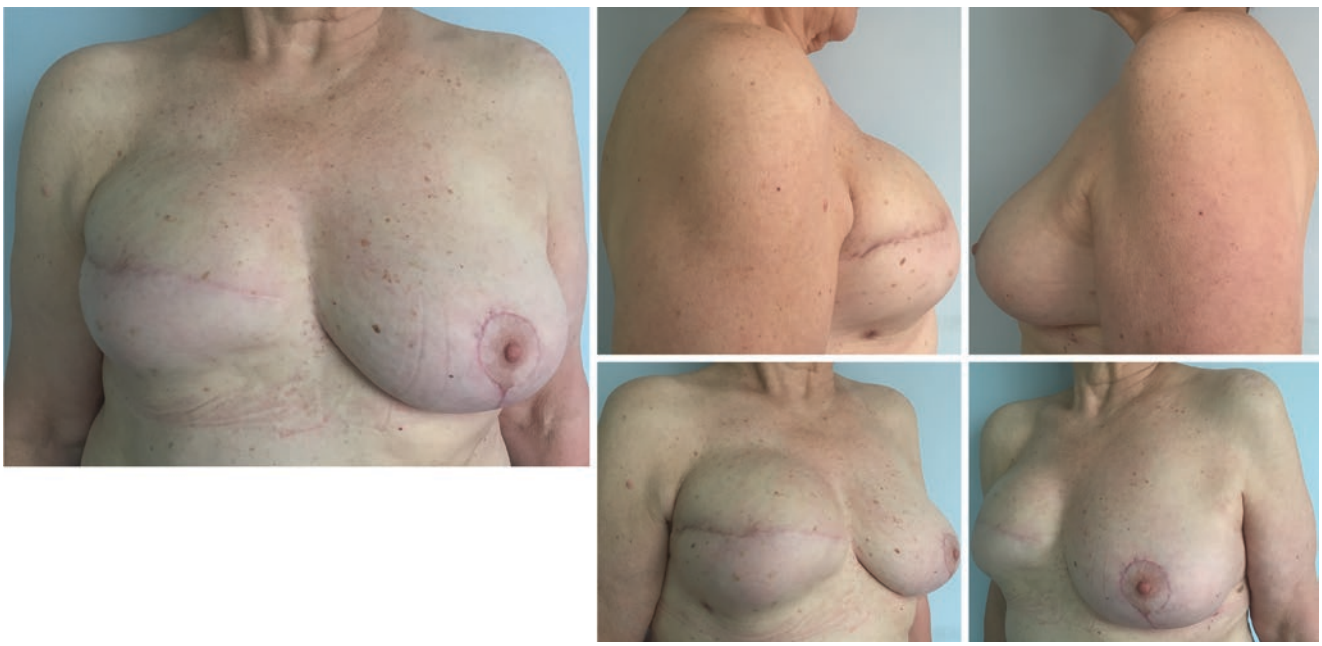


Fig. 33.6 Final outcome after definitive anatomical implant in the right breast and contralateral mammoplasty

33.4.2 Surgical Markings

Afterward, lines are drawn on the patient's chest to assure the correct understanding of the anatomic conditions. There should be drawn a median line from the sternal notch to the xiphoid appendix, and the inframammary fold should be placed at the same height and shape of the contralateral breast.

33.5 Surgical Technique

33.5.1 Before Skin Incision

In the operating room, patient is placed in supine position, keeping her arms parallel to the trunk. The operating table must be set in a way the patient can be placed in a 90-degree position, i.e., sitting, at the end of the procedure.

33.5.2 Skin Incision and Scar Excision

In cases of autologous flap delayed reconstruction, it is possible to remove part of the mastectomy flap, in order to replace that for the flap's skin and to shape the new breast. But with implants, the incision should be most of the times in the preceding mastectomy scar and, if possible, in the pectoralis major muscle. This technical detail allows for a safer suture of the prosthetic pocket in two layers, namely, the muscular and the cutaneous layers. Incision with either partial or complete removal of the scar is chosen based on three clinical situations:

- Wide scar with a good amount of skin in the mastectomy flap—in this case the exeresis of the scar is indicated.
- Narrow scar with little tense flap—here it is not necessary to remove the scar.
- Wide scars without much skin when it has already been decided to use an expander—scar can be removed completely or almost completely but extra care must be taken when expansion is performed, as a too sudden distension could widen the scar again.

33.5.3 Operative Technique

Autologous flap reconstruction is described in other chapters in this book. After incising the skin, an inferior and lateral subcutaneous undermining must be performed in order to do the contour of the inframammary fold. This is required to set the prosthetic pocket, which can be located subcutaneously in this region or under the serratus muscle, in case the skin or the adipose subcutaneous tissue in the inferior lateral region is too fragile. As a result of this maneuver, one can see the lateral edge of the pectoralis major muscle, which is then lifted to set the submuscular pocket. This pocket can be made via a digital undermining in the upper portion, where no perforating vessels are found. In the inferior and medial regions, a light retractor is required so that the efficient hemostasis of large internal mammary pedicles found in this region is performed. The pectoralis major muscle then must be completely detached from the costal plan about 4 or 5 cm above the medial extremity of the inframammary fold. This dissecting procedure is mandatory so that a nonaesthetic movement of the implant can be prevented when the pectoralis major muscle contracts. Preparation of the inframammary fold demands great technical attention, as it is an anatomic landmark crucial to the long-term aesthetic result [5]. There are two possible variants:

- Without an upper abdominal skin flap—It is used in cases either when there is great elasticity of the skin, which allows the insertion of a definitive prosthesis, or, if a decision has been made for a reconstruction in two surgical steps, of a temporary expander. In such cases, the subpectoral dissection must reach no more than the inframammary fold level, and then an incision into the aponeurosis of the rectus abdominis muscle must be performed to achieve a better projection of the lower mammary pole. There is no need for an undermining maneuver lower than the projection of the inframammary fold; otherwise, the prosthesis might end up being placed below the inframammary crease, producing asymmetry.
- Using an upper abdominal skin flap—This autogenous tissue reconstruction technique is recommended for those cases in which a definitive implant is applied and the skin flaps from mastectomy are not very elastic. A rectus abdominis muscle aponeurosis (made according to the projection of the inframammary fold) can be used if there is good elasticity of the skin in the upper abdominal area (just below the inframammary fold). The subpectoral dissection must reach the inframammary fold level followed by incision of the undermining of the supra-aponeurotic region 2–3 cm below the inframammary fold. A cutaneous advancement flap can be easily performed if the patient is placed in a semi-sitting position. The inframammary fold is reconstructed with spread stitches of nonabsorbable thread, suturing the superficial aponeurosis at the upper limit of the aponeurosis of the rectus abdominis muscle medially and laterally at the serratus muscle.

After the prosthetic pocket is set up, an internal irrigation is performed with either pure or with an antiseptic product-added saline solution. At this point, rigorous skin cleaning and change of gloves of the whole team before contact with the implant is mandatory. Such care helps to reduce the risk of microcontamination of the implants and therefore reduces the risk of postoperative infection or the formation and development of a peri-prosthetic capsule [25]. The implant, i.e., either the definitive implant or temporary expander, is carefully inserted into the prosthetic pocket.

Finally, a tubular multiperforated aspirating drain is inserted into the prosthetic pocket as a safety measure. Then, suture is done in two plans. The first suture is done in the subcutaneous tissue with absorbable monofilament stitches of 3-0, and the second is an intradermal cutaneous suture with absorbable monofilament stitches of 4-0.

33.6 Post-Operatory Care

Some surgeons apply a dressing with elastic straps, making a moderate compression for 3 days. Others choose a lighter dressing with no compression and also advise the patient to

wear a sports-type bra, medium compression, right on the first postoperative day. This second option allows an easier control of a possible postoperative hematoma and avoids risks of allergy and cutaneous lesions that might occur when adhesive elastic straps are used. The drain is removed when the drained fluid is serous and its volume is lower than 50 cc in the past 24 h. If a temporary expander is chosen, an expansion with a variable volume of saline solution is the usually recommended each 3 weeks. The correctly instilled volume should not cause tightness or erythema or disrupt the patient's comfort or skin quality. As the aim of the expansion is to surpass the quality of a one-stage definitive implant, an augmentation of 25% is needed to achieve this purpose, with ideal skin drape and recoil [5].

33.7 Association with Fasciocutaneous Thoracodorsal Flap

This technique was initially described by Holmstrom [10], who advocates the use of a rotational fasciocutaneous thoracic dorsal flap to improve the projection of the lower pole of the reconstructed breast. This technique can be applied in those cases of an oblique mastectomy scar, and the graft must be grounded on epigastric vascular pedicles, which cross the anterior aponeurosis of the rectus abdominis muscle. The flap must be designed with two thirds of the base above the future inframammary fold and a third below. After the preparation of the fasciocutaneous flap, an upper rotation of the flap is performed, and the donor zone is covered with the inferior rotation of the lateral triangular flap together with the advancing of the upper abdominal skin flap. The implant is inserted below the pectoralis major muscle in the upper internal region and below the flap in the inferior lateral region. This technique is not routine due to the vascular fragility of the flap. It can be used when applying more complex techniques such as when the latissimus dorsi or the transverse rectus abdominis myocutaneous (TRAM) flaps are contraindicated.

33.8 Complications

Complications related to breast reconstruction of any type can be classified into immediate (until 2 months after the surgery) or secondary (after the aforementioned period) [5]. The most frequent complications comprise hematomas, seromas, infection, flap necrosis, and capsular contracture. Capsular contracture rates may be lessened by the use of implants with a textured shell rather than a smooth shell, by placement of the implant in a submuscular rather than subcutaneous location, and by avoiding the use of this technique in women who need radiotherapy [26, 27]. Obesity, diabetes, age older than 65, smoking, and hypertension are risk factors for complications following breast reconstructions [28, 29].

Conclusions

Delayed breast reconstruction can achieve satisfactory cosmetic outcomes with low rate of complications. Temporary expanders and implants are surgical procedures that represent minor risks and, sometimes, can even be performed under day surgery. Overall, this is the most used technique due to its practicability, lower risk of complications than musculocutaneous flaps, and satisfactory aesthetic outcomes with the various anatomic implants available nowadays. Patients who were previously irradiated are better for autologous flaps or lipofilling.

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Immediate Breast Reconstruction (Direct to Implant)

34

Mario Rietjens, Pietro Loschi,
and Leonardo Pires Novais Dias

34.1 Introduction

The treatment of breast cancer until the late nineteenth and beginning of twentieth century was highly mutilating for the patients, in order to have some possibility of cure [1]. The standard surgery, at that time, removed large amounts of skin and thoracic muscles [2], combined aggressive radiotherapy, and determined a significant tissue degradation. With all these sequelae due to the treatment, it was practically impossible to propose any type of technique for breast reconstruction. The evolution of our biological knowledge of the breast cancer [3, 4], in association with screening programs all around the world, provided an initial phase diagnosis [5–10] and consequently concedes a de-escalation of the mutilating treatment [11–21]. These factors contributed to preserve the patient's quality of life [22–24].

The development of a non-mutilating mastectomy, as skin-sparing mastectomy or nipple-sparing mastectomy, was the main factor to evolve indications of direct-to-implant reconstructions (DTIRs) [14, 15, 17, 18, 20, 21, 25–44]. The conservative mastectomy represents the main step on surgical technique by preserving more overall skin and surface area to place an implant and by helping to avoid some of the flattening associated with closure of skin-sparing incisions [30, 40, 41].

The implantable devices evolution was also very crucial to increase indications to DTIR. The first breast silicone implant was used in 1960, and since then, the medical industry invested massively on research and innovations, and the surgeon today has an arsenal of possibilities to use, especially prosthesis and meshes. There were improvements on several points: less capsule contracture, more resistant and durable materials, and better cosmetic results concerning the anatomical implants with different models, shapes, and dimensions [1, 28, 39–41, 44–62].

The aim of this chapter is to give an overview about patients and implant selections in cases of DTIR, focusing in surgical planning, technical aspects, and complication management.

34.1.1 Indications

The technique indications are related to two main variables: breast anatomy (volume and shape) and tumor characteristics (size, local extension, and muscles or skin infiltration) [17, 19, 63–68]. Following the patient individuality, different patterns of mastectomy can be applied.

34.1.1.1 Small or Medium Breasts

Nipple-sparing mastectomy: in cases of breast with minimal ptosis, small peripherally located tumors with nipple-areola complex distance >2 cm, and negative subareolar duct margins. In selected cases, larger tumors can be submitted. Contraindicated in patients with inflammatory breast cancer, clinical involvement of the nipple-areola complex, nipple retraction, Paget disease, bloody nipple discharge, or multicentricity (Fig. 34.1).

Skin-sparing mastectomy: in cases of tumors closer to the nipple-areola complex, positive subareolar duct margins and previous scars that compromise the nipple-areola complex blood supply. On this last situation, the nipple-areola complex graft can be performed after the intraoperative subareolar duct evaluation. Contraindicated in patients with inflammatory breast cancer and extensive skin involvement by tumor (Fig. 34.2).

34.1.1.2 Large Breast

Conventional total mastectomy: in cases of large breast with ptosis grade III or IV and large tumors near the skin. It is possible to remove a large amount of skin over the tumor and have enough skin to perform a DTIR (Fig. 34.3).

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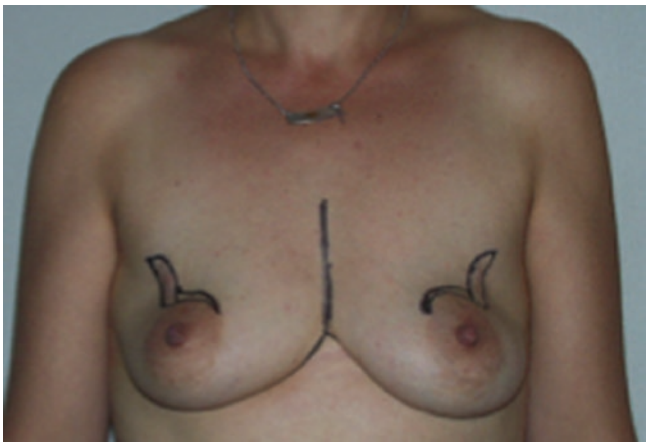

Fig. 34.1 Preoperative drawings for nipple-sparing mastectomy removing the previous biopsy scars

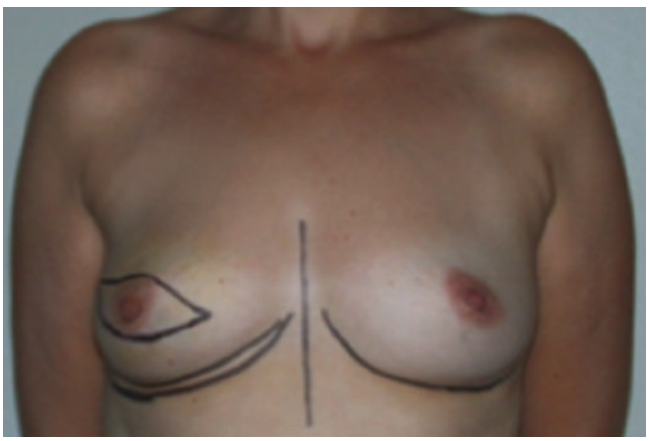

Fig. 34.2 Preoperative drawings for skin-sparing mastectomy removing small amount of skin

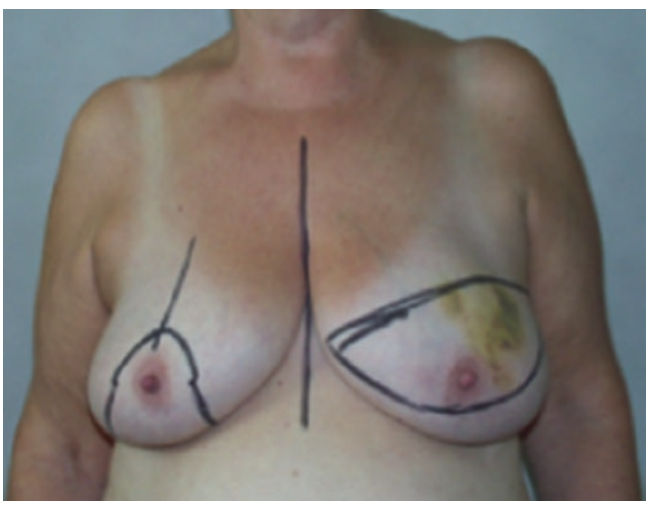

Fig. 34.3 Preoperative drawings for total mastectomy removing large amount of skin over the tumor located in the upper outer quadrant

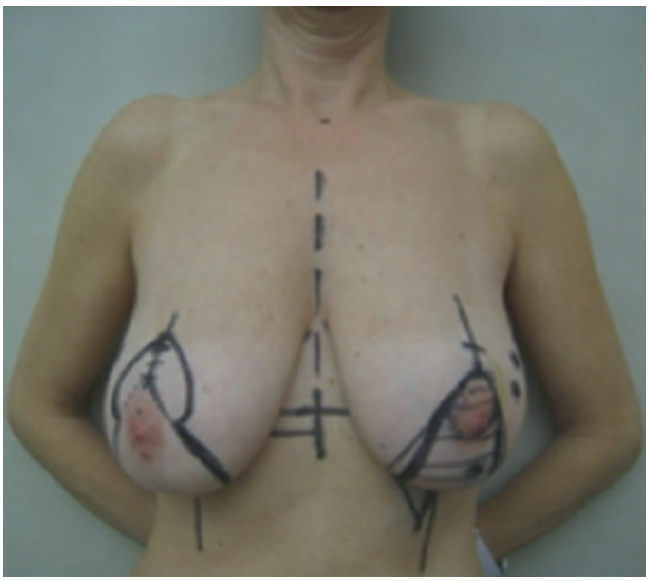

Fig. 34.4 Preoperative drawings for a left skin-reducing mastectomy with a modified "Wise pattern"

Wise pattern mastectomy: in cases of large breast with ptosis grade up to II (moderate). It is possible to remove an amount of skin in order to have an adequate skin envelope to cover the implant. Contraindicated in patients with inflammatory breast cancer and extensive skin involvement by tumor (Fig. 34.4).

Skin-reducing mastectomy: in cases of large breast with ptosis grade III or IV (advanced or severe, respectively). It is possible to reduce the amount of the skin envelope with a lower pole skin deepithelialization and use the dermis to help the implant cover. Contraindicated in patients with inflammatory breast cancer and extensive skin involvement by tumor (Fig. 34.4).

34.1.1.3 Relative Contraindications

Advanced disease (stage III or higher)
Need for postmastectomy radiotherapy
Significant medical comorbidities such as active smoking, obesity, or cardiopulmonary disease

34.1.2 Planning

Optimal management requires a multidisciplinary approach between oncologic and reconstructive surgeons, radiologists, pathologists, medical oncologists, nurses, and physiotherapists. This allows providers to coordinate cancer and reconstructive procedures with postoperative recovery and adjuvant treatment. Also, the oncological multimodality access has been associated with a reduction in breast cancer

mortality [69]. A caring relationship is crucial to patient satisfaction with the reconstructive process and must be established early [70]. Therefore, it is important to embrace the patient with all endearment since the first interview. In-hospital is highly recommended to examine the patient the day before the operation and explain again the complete surgical procedure in order to have the patient's consensus.

34.1.2.1 Preoperative Evaluation

History and physical assessment should focus on the following factors: stage disease, oncologic treatment plan, past surgical history, comorbidities, volume and shape of contralateral breast, body habitus, smoking story, and potential donor sites for autologous reconstruction.

A past medical history of radiotherapy on the same site or current disease extent for which radiotherapy is mandatory influences reconstructive options. Radiotherapy leads to fibrosis, which compromises the quality of the skin and underlying tissue, resulting in a higher incidence of complications from the reconstructive procedure, and may produce a less esthetically pleasing result [28–31, 59, 71–80].

Comorbidities such as obesity [31, 53, 59, 76, 81, 82], insulin-dependent diabetes mellitus [53, 76, 82], chronic obstructive pulmonary disease, smoking [31, 53, 59, 76, 81–83], and connective tissue disease may impact also the patient's reconstructive options. When poorly controlled, these comorbidities may increase the risk for complications such as impaired wound healing, reduced tissue perfusion, and infection [76, 84]. In addition, past surgical history of coronary artery bypass grafting (with use of internal mammary vessels) may limit reconstructive choices because of their adverse effects on the breast tissue blood supply.

Tobacco use also poses significant risks [31, 53, 59, 76, 81–83]. Due to the nicotine effect, as well as generalized tissue hypoxia as a result of carbon monoxide, this can increase the risks of tissue necrosis, delayed healing, and infection [85, 86]. For these reasons, avoidance of smoking is recommended for at least 4 weeks prior to surgery and 2 weeks following surgery.

The physical examination of the breasts includes an evaluation for volume, ptosis, asymmetry, scars, and the axilla examined for palpably abnormal lymph nodes. The abdomen and back are evaluated, taking note of scars and patient's personal distribution of excess skin and fat. Technical details such as the type of incisions following the oncological and plastic goals and the need of a contralateral breast correction are also determined at this moment. The patient's wishes regarding scar location, tissue sacrifice, postoperative recovery, and esthetic outcome are also important in guiding the reconstructive surgeon. Finally, the evaluation of bilateral mammograms, bilateral breast and axilla ultrasound, and, in specific cases, breast magnetic resonance is also indispensable.

Photographs of the patient standing and preoperative drawings are performed after admission at the hospital. During the preoperative drawings, it is important to do specific breast measurements as base width and height, projection, and pinch test (to evaluate the skin and subcutaneous thickness). With all these parameters, it is possible to calculate the range of models and implant size to be used.

34.1.2.2 Intraoperative Evaluation

The initial evaluation consists of verifying the skin and muscles integrity, soft tissue blood supply, and inframammary fold preservation. The implant is chosen using preoperative measurements, mastectomy weight, and contralateral breast modifications (reduction or breast augmentation). The use of sterile sizers is helpful to select the best implant aiming good symmetry.

The advent of real-time perfusion mapping and similar technologies represents an important aid for intraoperative planning. Some models predicting the risk for mastectomy flap necrosis have surfaced [87]. Although simple in concept, the surgeon's intraoperative judgment may be one of the more challenging aspects of DTIR and should be a focus of the perioperative decision-making process [29, 31, 88–90]. The unpredictable nature of the defect after oncologic resection is a particularly limiting factor, as implant size depends on the available soft tissue envelope.

In cases of soft tissue commitment and impossibility to perform the programmed surgery, the surgeon can convert it into a two-step reconstruction, using a tissue expander. In these cases, it is not recommended to perform the contralateral breast symmetry. A new evaluation will be done at the end of the expander inflation, and the surgery must be integrated with the timing of oncological treatment.

34.2 Implant Pockets

Until some years ago, I did not recommend the insertion of definitive prosthesis in the same location of the removed breast. The complication rates are very high, and if, unluckily, a small skin necrosi arrives or a scar dehiscence emerges, an exposure of the implant can occur, and the implant removal is necessary. Even if there are no postoperative complications, the normal healing around the implant with the capsule formation gives a very bad cosmetic results, with an aspect of "ball attach to the thorax" with an unpleasant aspect.

Nowadays, different possibilities of implant pockets are available, due to the new materials evolution [43, 91].

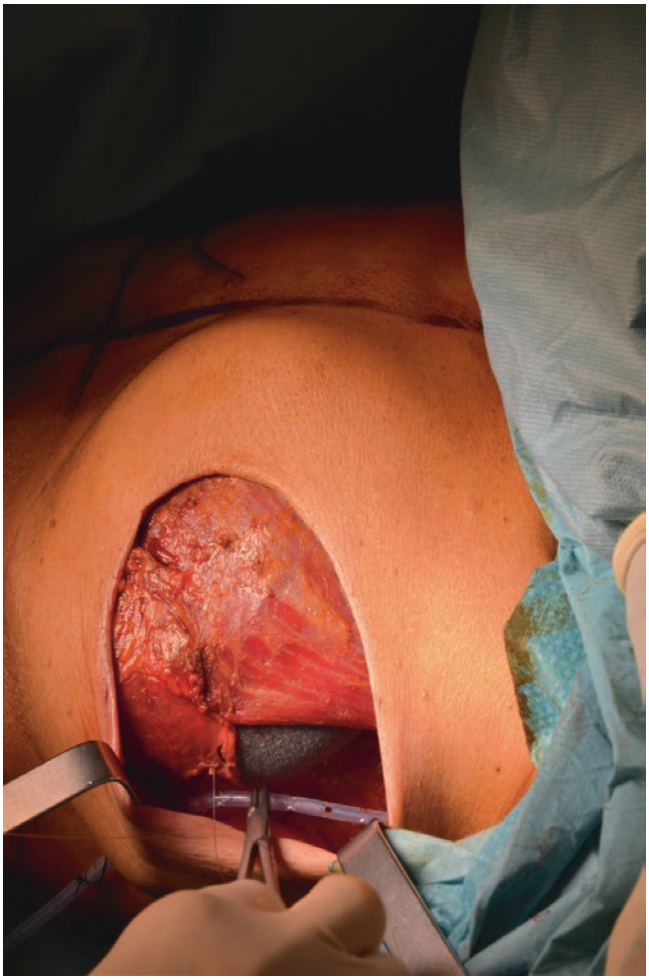

Fig. 34.5 Complete muscular pocket with pectoralis major muscle and serratus muscle

34.2.1 Total Muscular Pocket (Pectoralis Major and Serratus)

It was the first technique used in the past, when the mastectomy was less conservative. With this technique it was possible to cover completely the implant and avoid implant exposition in cases of skin necrosis. The cosmetic results were quite good when using small and round-shaped implants, but in cases of medium or large anatomical implants, it is difficult to cover completely the implant, and the cosmetic results are inferior because the lateral breast shape is compressed by the serratus muscle (Fig. 34.5).

34.2.2 Partial Muscular Pocket (Pectoralis Major)

This technique can be applied with safety in cases with a good lateral skin flap and when is possible to put all the mastectomy scar over the pectoralis major muscle, that one may have a good protection in cases of small skin necrosis or dehiscence. It is a very simple and quick technique and allows good results with anatomical or round implants. If the serratus muscle fascia is preserved during the mastectomy, it is also helpful to use it to cover partially the implant and avoid implant displacement, since it is the path of least resistance (Fig. 34.6).

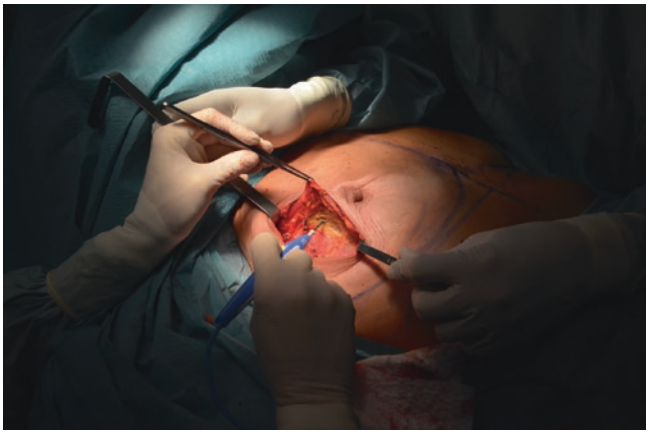

Fig. 34.6 Partial muscular pocket with only the pectoralis major muscle

34.2.3 Pectoralis Major Muscle and Synthetic Meshes

It is normally used in the outer lower portion of the breast, fixed to the lateral margin of the pectoralis major muscle and in the inframammary fold. The main indication of this material is mechanic, to maintain the implant at position and reduce the pectoralis major muscle retraction. The major problem with this material is the increase incidence of postoperative complications. The synthetic mesh is a low-cost alternative to biological matrices [35, 36, 39, 52, 57].

34.2.4 Pectoralis Major Muscle and Biological Meshes

The biological mesh is a collagen tissue matrix from which cell debris, DNA, and RNA are removed by complex proprietary process, leaving behind an acellular matrix [92]. This immunologically inert biological implant serves as scaffold necessary for tissue ingrowth, angiogenesis, and regeneration and can be integrated completely in few weeks. It is normally used in the outer lower portion of the breast, fixed to the lateral margin of the pectoralis major muscle and "wrapped" over the implant, not fixed to the inframammary fold. The major goal is to create a new tissue surface to cover the implant and reinforce the thickness in the outer lower portion of the breast [34, 53, 55, 61, 93, 94]. The material is

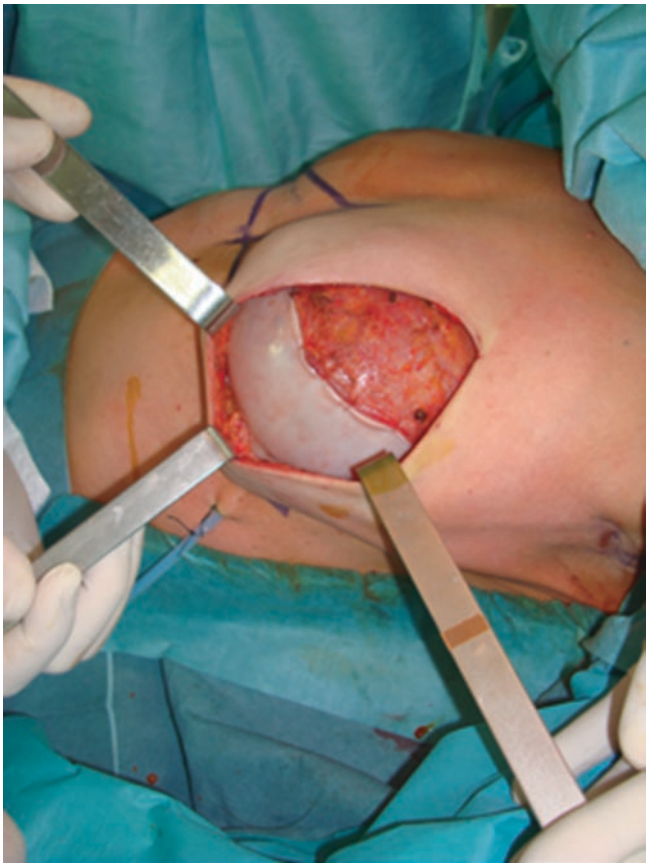

Fig. 34.7 Mixed pocket with pectoralis major muscle and ADM in lower outer quadrant

still very expensive [95, 96], and in our institute, the main indications are in cases with previous radiotherapy or complete inferior detachment of the pectoralis major muscle and rectus abdominis fascia. Using these material in these indications, it is possible to avoid a muscular flap, and it is also possible to reduce the capsule contracture after an implant breast reconstruction in cases with previous radiotherapy [55, 59, 61, 62, 97, 98] (Fig. 34.7).

A recent meta-analysis demonstrated that the risk of implant loss was not significantly affected by whether or not ADM was used. This result may be surprising given that the risk of infection, seroma, and mastectomy flap necrosis were significantly elevated in the ADM cohort. Although the use of ADM raises the risk of other complications including infection, they may not be that serious and can be clinically controlled without causing the implant removal [61].

34.2.5 Only Biological Mesh

Recent developments of this new technique showed that it is possible to do a breast reconstruction with implant without any muscle. The implant is completely covered with the biological mesh and implanted in the subcutaneous space. The cost is still very expensive and remains the main problem with this kind of technique [99, 100] (Fig. 34.8).

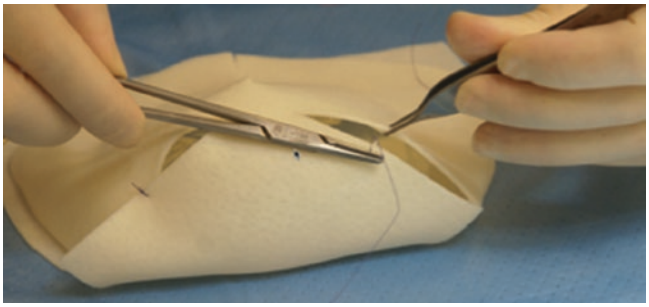

Fig. 34.8 Braxton® device consists in an ADM pocket with the implant inside, and it will be positioned in the subcutaneous space

34.3 Contralateral Breast Management

The goal of DTIR is an immediate reconstruction with definitive implant avoiding a second operation [1, 40]. For this reason, a contralateral breast mastoplasty is necessary in order to get an optimal symmetry [101–104] (Figs. 34.9, 34.10, 34.11, and 34.12). There are four main options for the contralateral breast.

34.3.1 Mastopexy

The DTIR allows a reconstructed breast without ptosis; for this reason, the contralateral ptosis should be corrected in order to get a good symmetry. Several techniques are available depending on the ptosis degree.

34.3.2 Reduction Mammoplasty

The DTIR is limited for the size of the breast to be reconstructed, because the maximum breast implant size available is around 700 cc. For this reason, in cases of large contralateral breast, a reduction mammaplasty should be performed in order to get a good symmetry. Several techniques are available depending on the breast shape, dimension, and ptotic degree.

34.3.3 Breast Augmentation

This technique is proposed for patients with small breasts and desiring a breast augmentation. The implant used is

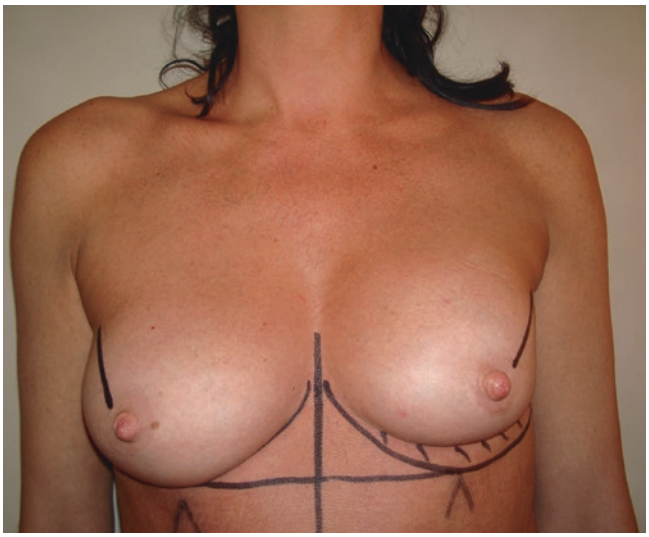

Fig. 34.9 Preoperative drawings for a bilateral nipple-sparing mastectomy with a radial incision and a DTIR

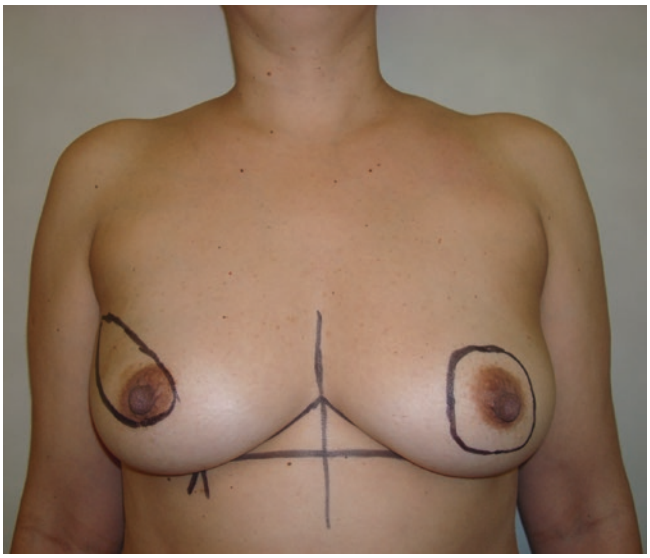

Fig. 34.11 Preoperative drawings for a right total mastectomy and DTIR and left periareolar mastopexy.

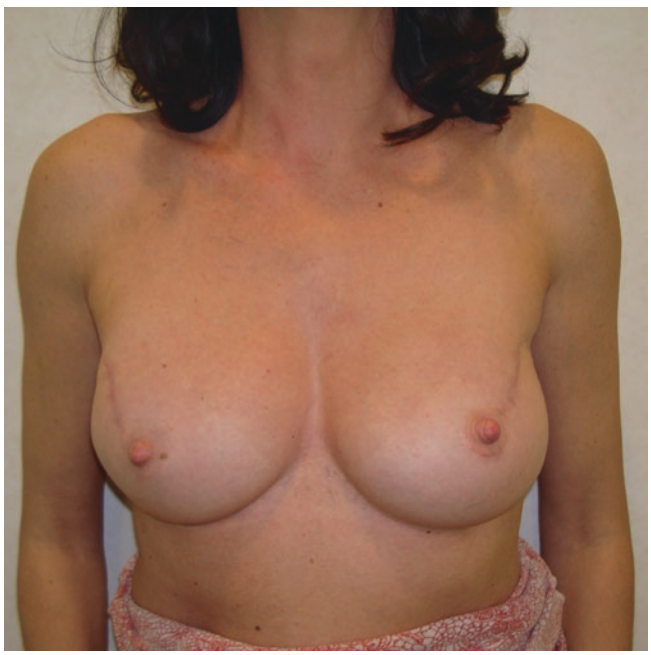

Fig. 34.10 Final results after 6 months

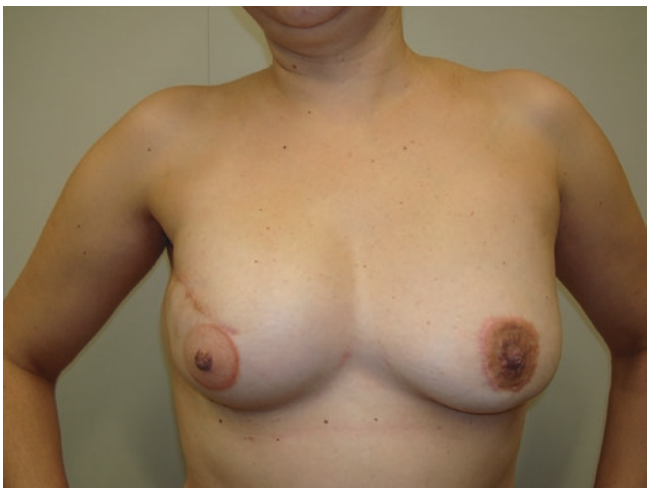

Fig. 34.12 Final results after 12 months.

frequently as a round-shaped implant positioned under the glandular tissue or under the pectoralis major muscle (dual plane technique).

34.3.4 Prophylactic Contralateral Mastectomy

This technique increased the indications in the last years after the possibility to diagnose a breast cancer familiarity with BRCA 1 and 2 genetic test [105]. Using the same technique and the same implant size and shape in both breasts, the breast symmetry is frequently optimal [106].

34.4 Technical Aspects

34.4.1 Cleaning and Arm Reposition

Once the mastectomy is finished, the skin is cleaned once again with chlorhexidine [107], and new sterile drapes are placed on top of the original ones. The arms are positioned along the body on arm boards, in order to promote a pectoralis major relaxation.

34.4.2 Skin and Muscle Evaluation After Mastectomy

The two main points are the coverage capacity, allowing the junction of tissue flaps without excessive tension on the suture, and the vascular supply of the mastectomy flaps that is evaluated by soft tissue thickness, color, and bleeding.

34.4.3 Pocket Dissection

The pectoralis muscle is elevated with a light retractor from lateral to medial; the lower muscle attachment is released with electrocautery from approximately 4–8 o'clock, preserving the rectus abdominis fascia, which is essential to have a complete pocket. The inferior dissection is performed until the inframammary fold level. The lower outer pocket dissection depends on the technical choice; the implant can be positioned subcutaneously, under the serratus fascia or under the serratus muscle.

34.4.4 Cleaning and Draining

The skin is cleaned once again with chlorhexidine, and internal pocket is irrigated with povidone solution [108]. One or two drains are inserted: one drain is in the submuscular pocket and, in cases of axillar lymphadenectomy, a second drain is placed in the subcutaneous space and axilla.

34.4.5 Implant Insertion

The operative team is also structured, so that there are no changes of surgical scrub technicians once the implant is opened until it is inserted in the breast pocket. The gloves are changed; only one surgeon handles the prosthesis and the implant is placed. The pocket is closed using monocryl 3.0 sutures.

34.4.6 Skin Closure and Dressings

The skin edges are trimmed and the skin is closed in layers with monocryl 3.0 and 4.0. Drains must be open at this time, to control the surgical site. Then Steri-Strips are positioned over suture site, and sterile gauze dressing is placed around the drains.

34.4.7 Post-op Cares

The patient stays in the hospital one or two nights. A sportif bra is placed prior to discharge from the hospital, after the prime dressing is pulled off, to help support the implant(s). Upon discharge, the drains, which are covered with and occlusive dressings, are maintained for 7–14 days, with removal determined by the amount and quality of their output, which should be less than 40 cc in 24 h.

34.4.8 Nipple and Areola Reconstruction

In cases of DTIR that the NAC was not preserved, the option of nipple-areolar reconstruction is proposed to the patients after 4 months. There are multiple techniques by which a nipple and areola can be recreated. Surgical methods involve local tissue rearrangement procedures or skin grafts [109]. These techniques are executed on day hospital, considering that it requires only local anesthesia. Once the projecting papilla has been created, the appearance of the entire nipple-areola complex can be enhanced by the use of tattooing. An alternative to surgical reconstruction of a nipple is three-dimensional tattoo. This is usually performed in a nonmedical setting by an experienced tattoo artist. The results have been excellent and patient satisfaction has been high.

34.5 Complication Management

34.5.1 Hematomas

It occurs usually in 1–5% [28, 29, 31, 55, 110] of the patients within 1–3 days after surgery. The symptoms are swelling of the breast and increasing pain that, sometimes, does not respond to pain reliefs. If the amount of blood is small, no treatment is required if the drains are still in place, but if the collection is moderate or large, a surgical revision is necessary to remove the coagulated blood, clean the cavity e insert new drains.

34.5.2 Skin Necrosis

The risk of skin necrosis is higher in DTIR compared with tissue expander reconstruction [37, 110, 111]. It occurs around 1.25–26% [28, 31, 55, 112]. Conservative wound care and a second intention healing can be used if the implant is completely covered by the muscles [82, 112]. In cases of skin necrosis with partial muscular pocket, the risk of implant exposure and an implant removal is very high. In these cases, the skin edge necrosis can often be managed with debridement and closure under local anesthesia. If the necrosis is more severe, the implant may need to be downsized or changed to a tissue expander [76, 83].

34.5.3 Seroma

34.5.3.1 Immediate

In the days following the surgery, fluid can collect around the implant, causing pain or swelling, if it is not adequately drained or if the drains are removed before the body can reabsorb the lymphatic fluid. It occurs in 1.5–7.5% of the cases [29, 31, 55, 56]. Removal of larger seromas is recommended since they can become infected. Usually, the fluid can be removed carefully with a puncture guided by an ultrasound and does not require additional surgery [56, 76].

34.5.3.2 Delayed

The etiology remains unclear; one supposed cause can be due to an internal tissue irritation with the textured implant surface in cases of an intense physical effort. Another possibility is a focal infection outside of the breast (oral, urinary, etc.) that can stimulate a fluid production around the implant. In cases of small seromas, only an oral therapy with antibiotics and anti-inflammatory for 10 days can solve the problem. Otherwise, in cases of large seromas, it is also necessary to aspirate the liquid to do bacterial analyses (antibiogram) and also cytology, for eventual diagnosis of anaplastic large cell lymphoma (ALCL) [113, 114]. In cases of frequent seroma recurrences, it is necessary to do an implant revision with large capsulectomy and implant change.

34.5.4 Infections

In cases of DTIR, it is around 1–5.2% of the cases [28, 29, 55, 115], and the most common organisms are *Staphylococcus aureus* and *Staphylococcus epidermidis* [115–117]. The main symptoms include pain, cellulitis, swelling, and fever. Initially, the treatment is based on oral antibiotics or, if the infection is severe, on intravenous antibiotics until the clinical and laboratory exams maintain stable for 48–72 h; then recommendation is return to oral antibiotics for 1–2 weeks more [76]. In severe cases, antibiogram is strongly suggested to guide the treatment. The presence of gram-negative rods or methicillin-resistant *Staphylococcus aureus* is relative contraindication to salvage attempts based on the poor success rate. The risk factors associated with postoperative infections after IBR are chemotherapy, smoking, radiation therapy, intraoperative lymph node dissection, and larger breast size [116]. In cases of severe infections, without positive response with antibiotics, the implant removal is indicated, clean and drain the breast, and reevaluate the local conditions after 4 months, in order to plan another breast reconstruction [76].

34.5.5 Implant Exposition

The implant exposition can be observed after skin necrosis or wound dehiscence. It occurs around 2% [28]. The decision to try to conserve the implant depends on the time between the exposition and medical evaluation (more time = more possibility of implant contamination) and the skin quality and elasticity or previous radiotherapy. A case with good local conditions and short time of exposition is indicated to make a debridement, new suture under local anesthesia, and antibiotic therapy. In the contrary, in cases with bad local conditions or previous radiotherapy and large time of exposition, the best solution is the implant removal and revaluation after 4 months for a new technique of breast reconstruction.

34.5.6 Implant Rotation

The main problem is due to a poor adhesion between the implant and the capsule, and the reasons can be thin capsula, periprosthetic fluids, or double capsulae. It occurs around 0.9% [55]. An implant surgical revision is indicated with a large capsulectomy to create a new adhesion area; try to close death spaces and also consider to change the anatomical implant with a round-shaped implant.

34.5.7 Capsular Contracture

This is the main long-term problem after implant surgery, and the rates are very different in the literature [28, 29, 55, 56]. The characteristics are pain, hardness, and changes on breast shape. The classification of Baker is useful to guide the treatment on this situation, and usually capsulotomy associated with implant replacement is reserved for Baker III/IV. A number of factors may reduce the occurrence of capsular contracture. These include submuscular implant location, use of textured implants, and prevention of postoperative infection or bleeding. Radiotherapy is closely connected with capsular contracture, because of its effects over the soft tissue [28–31, 53, 56, 71–75, 77–80].

34.5.8 Implant Rupture

The recent implant rupture is not detected by clinical examinations; for this reason, our follow-up protocol for patients with implant is a bilateral breast ultrasound each year and a breast MRI at 10–15 and 20 years of implantation [118–120]. In cases of implant rupture, an implant surgical revision with capsulectomy is indicated; remove the implant and all silicone inside the capsula and change the implant. In cases of axillar lymph nodes augmented after implant rupture, lymph

node removal is indicated only in cases presenting a very large and painful nodes. The others lymph nodes slightly augmented can be followed, and it will become normal few months after the implant change.

Conclusions

There are a number of potential advantages to single-stage DTIR as opposed to a conventional two-stage implant reconstruction. One benefit is avoiding a second operation and the expansion period necessary for tissue expander/implant reconstruction, allowing a shorter time to final reconstruction [28, 30, 37, 40, 110]. It improves patient quality of life and reduces the inconvenience of frequent clinical visits [28, 30, 37, 40, 110, 121]. The direct-to-implant procedure may have a higher risk of postoperative comorbidities and failure compared with two-stage reconstruction, so patient selection is an essential issue combined with a good surgical team experience [28, 29, 37, 40, 41, 55, 58, 60, 76, 81, 110, 122–125].

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Breast Reconstruction with Tissue Expander and Definitive Implant Replacement

35

Thomas H.S. Fysh and R. Rainsbury

35.1 Introduction

For patients who are not suitable candidates for autologous breast reconstruction, the traditional approach has been a staged procedure, by first expanding the skin and chest wall musculature over a period of weeks and then exchanging the expander for a fixed volume implant. While this was conventionally a delayed procedure to be carried out once adjuvant treatments were completed, it is now routinely used in the immediate setting [1].

The use of silicone implants for breast augmentation was described as long ago as the early 1960s, but it was another decade before Snyderman published the technique for a rudimentary, single-stage immediate implant-based reconstruction [2, 3]. It was not until 1982 that Radovan first described formal tissue expansion after mastectomy followed by exchange for a fixed volume silicone implant [4]. Since then, the steady advances that have been made in implant technology and dermal substitutes, as well as the rising demand for reconstructive and oncoplastic breast surgery, have served to increase the popularity and improve the outcomes associated with two-stage, expander-based breast reconstruction.

35.2 The Rationale for the Two-Stage Breast Reconstruction

Each reconstructive option has its attractions, but while the aesthetic outcomes of implant-based reconstruction may be inferior to tissue-based techniques, high-quality series and national audits nonetheless report rising patient demand and high levels of satisfaction following this approach [5, 6]. Two-stage implant-based breast reconstruction is, in many respects, the simplest of options. As such, it has found favour where other approaches cannot be considered, either because of operative risk, resource limitations or patient preference.

35.3 Patient Selection and Relative Contraindications

Given that the maximum volume achievable in two-stage breast reconstruction is around 650 ml, the ideal patient is usually of slim to normal habitus or only mildly overweight. The pectoralis should be innervated and functioning, and the skin flaps should be healthy. Although contralateral symmetrising surgery is usually straightforward, it may be difficult to achieve significant ptosis with this approach, and some patients will go on to have a mastopexy or reduction either at the time of their reconstruction or later on.

Patients undergoing immediate breast reconstruction will usually be suitable for either a 'direct-to-implant' approach or an autologous tissue-based reconstruction with or without an implant. A two-stage approach, however, provides the patient and surgeon with more flexibility, and there are many examples of situations when it is an attractive option.

While the initial surgery is relatively simple and low risk, patients who choose to undergo two-stage breast reconstruction must be advised that they are likely to require adjustments or revision of their reconstruction at a later stage. Leading manufacturers of cohesive gel implants generally advise that after 10–15 years, more than half the number of implants will have been replaced. Furthermore, several high-volume case series have shown that almost half of patients who have a planned 'two-stage' breast reconstruction actually go on to have three or more procedures [7, 8]. It is also clear from these and other reports that patients who have postmastectomy radiotherapy are particularly at risk of complications and have poor aesthetic outcomes. But while radiotherapy is often regarded as a contraindication to implant-based breast reconstruction, this view is currently being questioned as discussed below.

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35.4 The Operative Approach

35.4.1 Marking Up

With the patient standing up, her feet a comfortable distance apart and her shoulders relaxed with arms by the side, the midline is marked, starting at the sternal notch. The breast meridian is marked on the normal side, as is the inframammary fold (IMF), with its most dependent point marked in the midline. The planned IMF is then marked on the operative side, with the most dependent part marking the intersection of the planned new breast meridian. The upper and lateral borders are matched with the normal side, and so a new breast 'footprint' is marked and the base width noted. Some manufacturers provide transparent plastic templates for this purpose. The skinfold thickness is taken away from the base width to give an expander base-width measurement. The exact choice of the expander will depend on the manufacturer and familiarity with use, but in general, the choice of devices is smaller than that of the fixed volume implants. The height of the device is only relevant in anatomically shaped 'adjustable implants', which are usually more expensive than 'true' tissue expanders and used in situations where they are unlikely to be exchanged (their use is discussed below). As a rule, we suggest ordering two expanders of the measured base width, two of the size above and two of the size below, which allows for size discrepancy and accidental contamination or damage.

35.4.2 Antisepsis Measures

- Screening for methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* (MRSA and MSSA).
- When screening is positive, implement a clearance regimen (e.g. nasal mupirocin and chlorhexidine body washes) for 5 days before surgery.
- Where possible, a 'clean air' theatre is used (e.g. laminar flow).
- Passage of staff in and out of theatres is restricted.
- Single dose of IV antibiotics should be administered on induction according to local policies, to reduce the risk of post-operative infection, and repeated in procedures lasting more than 4 h.
- The skin should be prepared with a single wash of 2% chlorhexidine in a 70% solution of isopropyl alcohol and allowed to dry. Povidone Iodine is less effective [9].
- The pocket is prepared as much as possible prior to implantation, even with the use of an ADM.
- All operating staff put on a second pair of gloves prior to opening the expander (or wear two pairs, taking the outer pair off at this stage).
- The authors favour a 'minimal or no touch' technique, whereby the expander is opened immediately before implantation and bathed in a betadine/antibiotic mixture [10] or aqueous chlorhexidine. High-quality evidence confirming that this measure reduces implant loss is lacking and there is some concern that betadine may cause local tissue trauma, increasing capsule formation; some favour saline washes only.
- Contact between the skin and the implant should be avoided.
- Time between implantation and skin closure should be minimal; consultants should determine how much time to allow trainees with this in mind since operating time is directly related to surgical site infection rates.
- The pocket is thoroughly washed prior to implantation, with special attention given to removing loose fat and necrotic tissue, which could provide a nidus for infection.
- The use of special devices such as plastic sleeves to deliver the prosthesis into the pocket is largely untested, but is conceptually attractive as they allow for a true 'no touch' approach.

35.4.3 Intraoperative Technique

The procedure is undertaken under general or regional anaesthesia such as paravertebral blockade and sedation, with the patient in the supine position and arm abducted.

In the delayed setting, the incision is made usually via the previous mastectomy wound, but there is the opportunity to redefine the new IMF if necessary at this stage. Alternatively, this can be considered at the time of implant exchange. The techniques for redefining the IMF are described below; this is generally more important in patients who have undergone chest wall radiotherapy, since nonirradiated skin should expand without difficulty. It is preferable to excise as much irradiation-damaged and scarred skin as possible at this stage.

In the 'immediate reconstruction' setting, the mastectomy is most commonly performed using a skin-*reducing* pattern, since a total skin-*sparing* approach is usually more appropriate for a 'direct-to-implant' reconstruction. While it is technically possible to retain the nipple in a skin-reducing pattern, the vasculature of the nipple-areola complex is extremely precarious, and patients must be advised of the greater risk of ischaemic complications, especially when adjuvant therapies may be required. The template for the skin-reducing approach is a modified 'Wise' pattern, whereby the T junction is raised a little and the angle of the apex made more acute than for a breast reduction. As such, the final incision takes on more of a 'Mercedes' sign appearance than the classic 'inverted T'. The surgeon's aim is to be able to drape

the skin over the reconstruction 'mound' such that it is not under tension but in such a way that it remains 'smoothed out'. In both the immediate and delayed setting, the skin is elevated from the chest wall musculature about 1–2 cm beyond the superior and lateral boundaries of the planned breast 'footprint'. Care should be taken to preserve the perforating intercostal branches medially, and some surgeons like to mark these preoperatively using a handheld Doppler device.

The pectoralis major is then elevated and detached from the chest wall at its costosternal origin as far as the level of the planned maximum breast projection (usually the fourth intercostal space). It is crucial NOT to over-dissect the submuscular pocket superiorly or laterally. It is all too easy and tempting to do so, since this is a natural tissue plane, but the expander will follow the path of least resistance once in situ and will tend to migrate in this direction.

In its lower pole, the expander must be covered either with an ADM and/or chest wall fascia/musculature. Using the former approach, the IMF and lateral border can be easily defined by carefully suturing the lower border of the ADM to the anterior rectus sheath inferiorly and to the serratus fascia laterally; indeed this is the main reason for using an ADM in this context. Since the ADM itself cannot be expanded, a small a piece as possible should be used and attached to the pectoralis muscle with interrupted absorbable sutures. At the time of implant exchange, the incorporated ADM can be incised radially to improve its compliance and shape. When using the skin-reducing approach, the lower pole breast skin can be de-epithelialised, forming an 'inferior dermal' or 'lipodermal' sling. This avoids the cost and risks associated with ADMs and is sutured to the pectoralis to provide implant cover (Figs. 35.1, 35.2, 35.3 and 35.4).

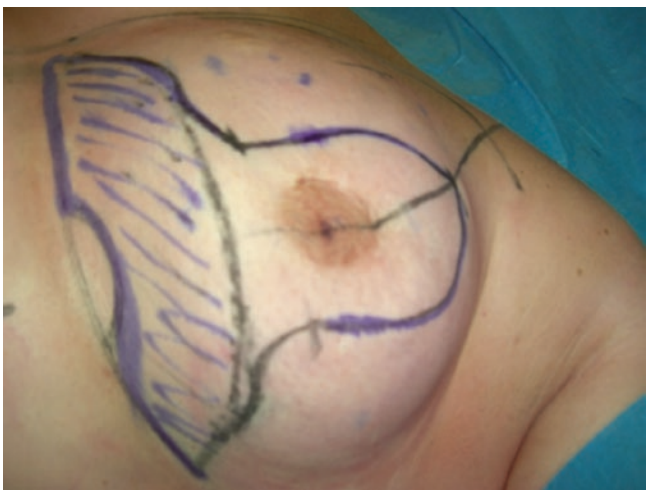

Fig. 35.1 Mark-up for skin-reducing mastectomy and inferior lipodermal sling with Becker™. *Inferior shaded area* is de-epithelialised and skin template cut quite loosely compared to a cosmetic breast reduction

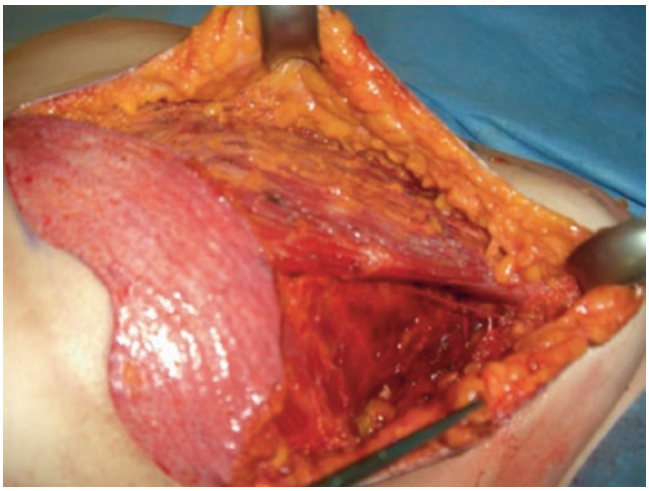

Fig. 35.2 The de-epithelialised inferior sling is sutured to pec major superiorly and serratus laterally to form the expander pocket. The skin is draped over the top without undue tension

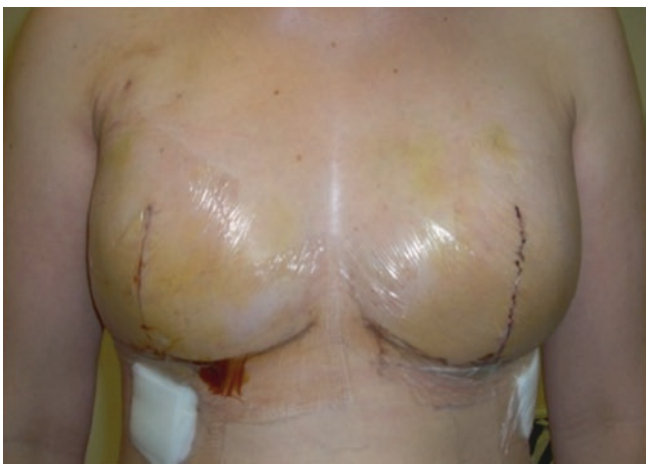

Fig. 35.3 Early post-operative appearances (bilateral procedure), Becker™ adjustable implants 70% filled

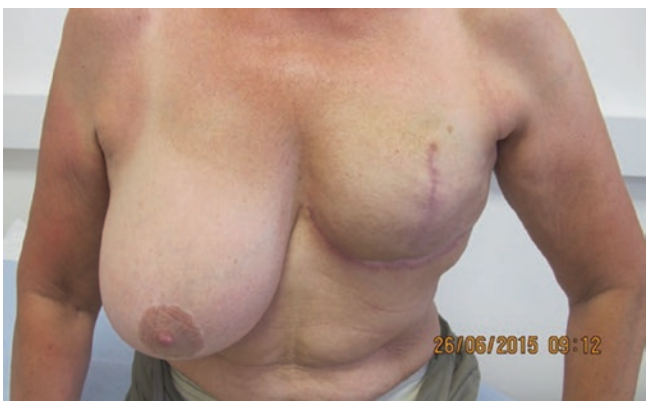

Fig. 35.4 Three months post-operative left skin-reducing mastectomy and lipodermal sling with Becker fully inflated (with prior right augmentation mastopexy many years ago). This patient unexpectedly required postmastectomy radiotherapy and the implant extruded 12 months later

It is the authors' preference to use a single suction drain at closure, which can be fed into the expander pocket and through the skin via the mastectomy pocket. It is important for any dead space to be drained long enough for the ADM or lipodermal sling to incorporate into the skin envelope. Typically, this means the drain is left for 1–2 weeks. As with any immediate reconstruction, it is preferable to stage the axilla beforehand or intraoperatively. This not only avoids unnecessary early reoperation, risking exposure of the expander, but may also influence the timing and type of any reconstruction. There is a lack of evidence to demonstrate that skin necrosis rates are higher when accessing the axilla via the mastectomy incision. Some surgeons prefer to minimise traction and trauma to the skin envelope by accessing the axilla through a small separate incision, thereby keeping the axillary and mastectomy pockets separate. If required, the use of high-intensity illumination and careful exposure with atraumatic retractors can provide excellent axillary access even through a small circular mastectomy incision.

35.5 Radiotherapy in Two-Stage Implant-Based Breast Reconstruction

It has long been held that postmastectomy radiotherapy is a relative contraindication to two-stage implant-based breast reconstruction. Ideally, it would be preferable to replace the irradiated skin with fresh tissue from elsewhere. Sometimes, however, this may not be an option because patients are not willing or able to consider autologous reconstruction. When two-stage implant-based breast reconstruction is undertaken either after chest wall radiotherapy or before planned chest wall radiotherapy, it must be on the understanding that the rates of all complications, including reoperation, implant extrusion and reconstructive failure, are much greater, often quoted as high as 50% [7, 11]

35.5.1 Patients with Planned Radiotherapy

In the immediate setting, it may be appropriate to use a two-staged approach to implant-based reconstruction in patients for whom the adjuvant treatments are unclear. This is sometimes referred to as 'skin-banking' or a 'delayed-immediate' approach. Common reasons for adopting this approach include:

- When it is unclear preoperatively whether or not patients will require or accept postmastectomy radiotherapy. Few would disagree that it is preferable to avoid any implant-based breast reconstruction when radiotherapy is being considered. The value of 'skin banking' is this context is questionable since healthy skin can usually be transferred along with an autologous flap later, and so the simpler approach of a well-executed, IMF-based mastectomy is often the preferred option.
- For patients who have not yet decided what kind of definitive reconstruction they would like, but who will not accept being flat chested at any point.
- For patients who need cancer surgery soon and do not want to be flat chested, but who can wait for definitive reconstruction for other reasons, such as buying time to stop smoking, attending important personal engagements or when there is lack of access to specialist services.
- For patients who have had a previous augmentation and who need the existing implant to be removed as part of their cancer surgery. In these patients, the implant pocket (or parts of it) can usefully be retained, but often needs to be expanded to make up for the volume lost to the mastectomy.

35.5.2 Adjustable Implants Versus Tissue Expanders

Most tissue expanders are constructed from a solid state silicone shell surrounding a saline chamber that is accessed via an integrated metallic port on the anterior surface of the device. Most ports have a magnetic location system, which allows the clinician to cannulate the port transcutaneously without sonography. They are relatively cheap but are not designed to be left in situ. Moreover, the integrated port means that they are often unsuitable for patients likely to require MRI scanning or radiotherapy.

Adjustable implants such as the Becker™ expander series (Mentor, Johnson and Johnson) can provide an elegant solution for patients who are suitable for implant-based reconstruction but who are not suitable for either the "direct-to-implant" approach or in whom a two-staged "exchange approach" may not ultimately be necessary. These implants contain a variable volume of cohesive silicone gel (between 25% and 50%, depending on the type) with an adjustable inner saline chamber accessed via a remote subcutaneous injection port. Typically the port is removed under local anaesthetic, once the final volume has been achieved, and the adjustable implant is left in situ without being exchanged. These gel/saline implants are more expensive than traditional saline expanders or fixed volume gel-filled implants, and so their use should reflect the likelihood that they will remain in situ. In one case series reporting the outcome of >300 Becker™, 74% remained in situ >5 years following implantation, avoiding the cost and morbidity of exchange for a fixed volume device [12]. Examples of patients who might be suitable for an adjustable implant include:

1. Those for whom postmastectomy radiotherapy is dependent on histopathology findings, but may not be necessary.

2. Those who require contralateral reduction simultaneously. Since shape and volume match in such patients is difficult to predict, final adjustments may be needed.
3. Patients in more remote communities where access to a large implant bank is not possible.
4. Patient groups with poorer-quality skin flaps, but who would otherwise be suitable for the 'direct-to-implant' approach (such as the growing number of older patients requesting breast reconstruction). In such women, the pressure on the skin can be easily reduced if necessary without exposing the patient to further surgery (Figs. 35.5 and 35.6).

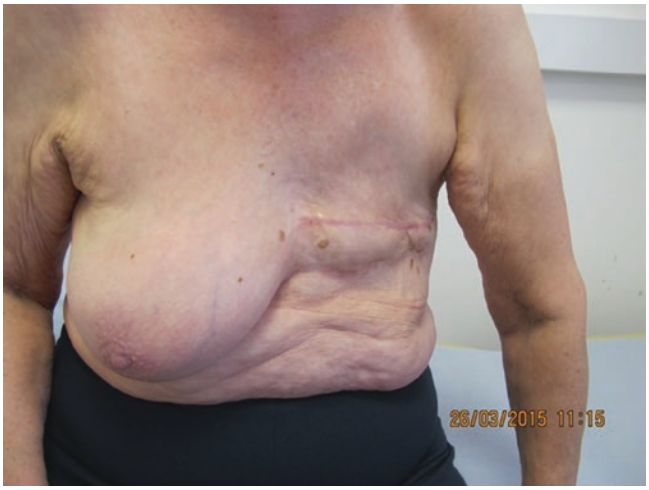

Fig. 35.5 78 year old with prior left mastectomy requesting reconstruction (preoperative). A single operation was desirable, and so a Becker™ adjustable prosthesis was used with a non-biological ADM (TiLOOP™)

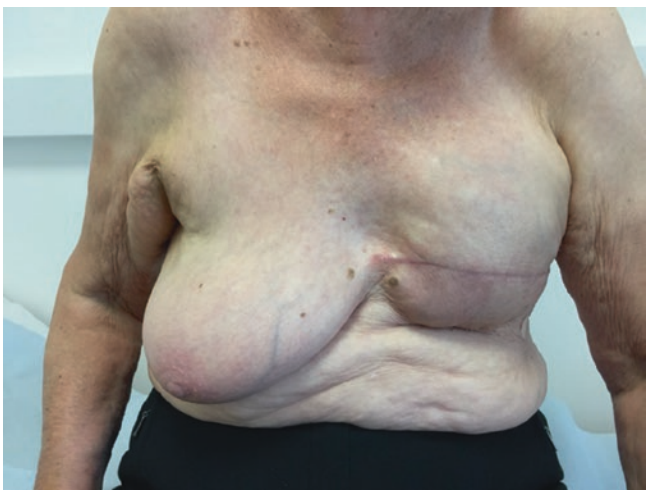

Fig. 35.6 Three months post-operative. Left delayed Becker™/ADM reconstruction in older patient. She underwent a single operation lasting approximately 1 h. She had good symmetry in a bra and declined contralateral reduction/mastopexy

5. Women with a very small (100–150 cc), somewhat flat breast mound who choose implant-based reconstruction but decline contralateral augmentation. The use of a slightly underinflated small (150–200 cc) Becker™ expander will achieve a breast shape which is as close as possible to the remaining breast. The base width is wider than a gel implant of equivalent volume, and the somewhat unnatural projection associated with smaller fixed volume implants can be avoided.
6. Patients undergoing bilateral immediate or delayed reconstruction after risk-reducing mastectomy who are uncertain about the most appropriate final volume. Becker™ provides considerable flexibility in relation to both volume and projection while avoiding the inconvenience and risks of subsequent exchange.

35.5.3 Timing of Radiotherapy in the 'Delayed-Immediate' Approach

For patients awaiting radiotherapy and not requiring chemotherapy, the expander is inflated fairly rapidly 2–3 weeks after surgery, provided wound healing is normal. This is usually straightforward because these patients do not require very much (if any) true skin expansion in the early stages due to the skin-sparing approach of the mastectomy. The aim is ultimately to 'overexpand' the skin envelope in order to preempt the fibrosing effects of radiotherapy. Most manufacturers of tissue expanders favour overexpansion and exchange to a fixed volume implant prior to radiotherapy. Overexpansion volumes are given in the manufacturers' leaflets, and while the actual tolerance tends to be well in excess of the advised overfill volume, most advocate an overfill of about 20% of the intended final volume.

Given that it is desirable to deliver postmastectomy radiotherapy within 4–6 weeks of surgery, this approach can lead to delays in order to accommodate hospitalisation and healing times. Although long delays can worsen outcomes, excellent loco-regional control is still achieved when radiotherapy is delivered within 8 weeks of surgery [13, 14]. Delay is seldom a problem since most patients with a disease profile warranting postmastectomy radiotherapy will also require post-operative chemotherapy. In this situation, expansion is carried out during chemotherapy, and exchange is carried out 3 weeks after the last cycle of chemotherapy and 3 weeks before the start of radiotherapy. These timing issues will become more common as the use of neoadjuvant chemotherapy continues to rise.

An alternative strategy is to delay implant exchange until *after* completion of radiotherapy. This may seem to be an attractive approach, as operative scheduling is simplified and irradiation of the final implant is avoided.

Recent evidence suggests however that this approach is associated with poorer outcomes, including high rates of capsular contracture and reconstruction failure and is best avoided [15].

35.5.4 Patients with Prior Chest Wall Radiotherapy

When patients present with an irradiated chest wall, but who have otherwise completed their adjuvant treatment, the basic approach to the reconstruction differs to that in nonirradiated patients. Only rarely will such patients end up having an implant-based reconstruction, and it is fair to say that these patients are extremely challenging. Most are suitable candidates for an autologous reconstruction, combined with an implant/expander or subsequent lipofilling if further volume is required, or an external prosthesis should be considered. For those wanting an implant-based reconstruction, several issues arise:

- The irradiated skin is less elastic, and the underlying pectoralis is often somewhat fibrotic, rigid and resistant to stretching. These factors combine to make expansion a much more challenging and time-consuming process. The number of expansions required will usually be greater, in smaller increments (e.g. 50 ml per expansion), with longer periods between each expansion.
- It is wise to be modest in terms of the final reconstruction volume. Attempting to use a large volume expander may preferentially depress the chest wall, causing a 'saucer' deformity of the rib cage, rather than expanding the skin. This can happen with smaller expanders too and should be anticipated and suspected in those patients who fail to achieve satisfactory projection despite numerous expansions.
- The expander will tend to migrate upwards or laterally towards the axilla, following the plane of least resistance. It is crucial to avoid over-dissection of the pocket in the first instance, although migration may still occur during the phase of overinflation (Figs. 35.7, 35.8, 35.9 and 35.10).
- Subcutaneous autologous fat grafting to improve the quality of irradiation-damaged skin has been shown to improve patient-reported outcomes in this context [16]. This may need to be repeated until visible improvement takes place, prior to delayed reconstruction.
- Nonirradiated skin will tend to expand preferentially compared to irradiated skin. Accurate placement of the tissue expander directly beneath the irradiated mastectomy flaps is important to avoid the creation of a 'double bubble' breast mound, due to differentially greater expansion of the unirradiated peripheral tissues.

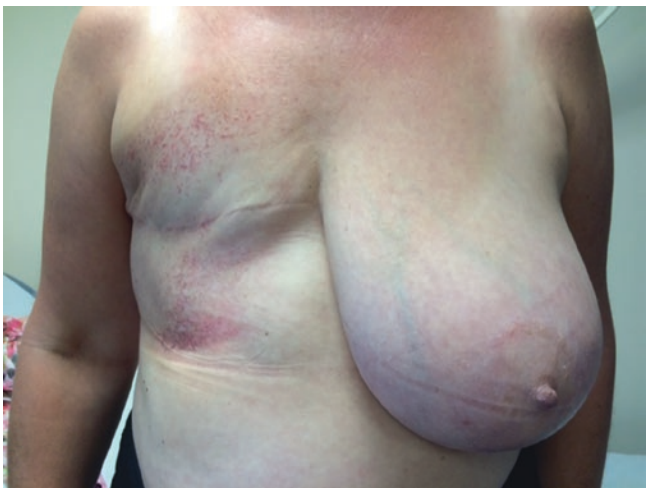

Fig. 35.7 50 year old requesting delayed reconstruction 5 years post-mastectomy and chest wall radiotherapy and axillary clearance

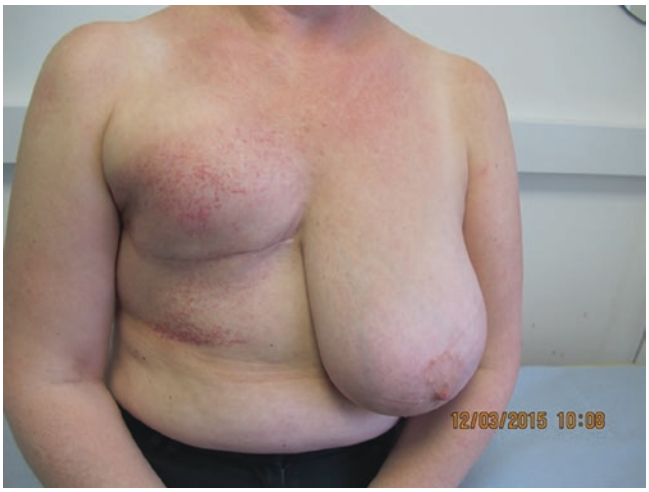

Fig. 35.8 Pt in Fig. 35.7, 4 weeks postoperatively. A planned LD was aborted intraoperatively, since the LD pedicle was destroyed by previous surgery and radiotherapy. An expander was inserted with and inferior non-biological ADM (TiLoop™) and filled with 60 ml initially

- Irradiated skin and muscle is unlikely to yield sufficiently to provide good projection even after expansion. This can be addressed in a number of ways
  - At the time of exchange to a permanent implant, a good volume of skin and subcutaneous fat can be recruited from the abdominal wall by dissecting beyond the IMF often as far as the umbilicus, in the plane of abdominal wall fascia. This 'abdominal advancement flap' can then be advanced into the lower pole of the new breast mound. It can then be secured in place with a line of sutures including the anterior rectus sheath and positioned to define the new inframammary fold. The imported, unirradiated abdominal tissue is then draped over the lower pole, providing ptosis and enhancing projection.

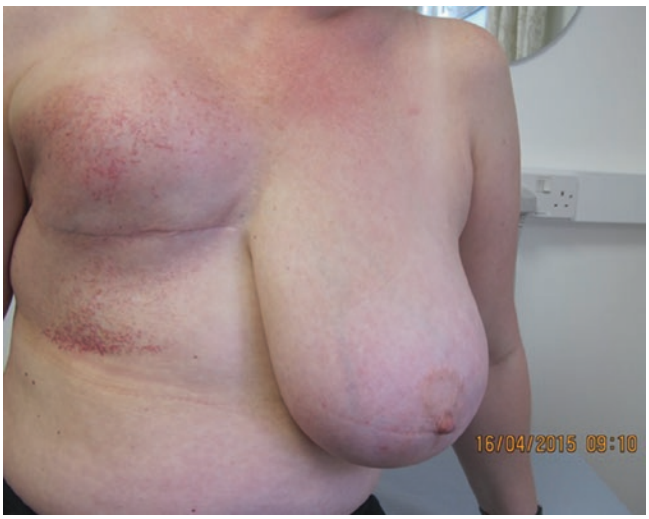

Fig. 35.9 Pt in Fig. 35.7. As expansion continues, the expander migrates along the path of elastic resistance, superolaterally. The radiotherapy damaged skin fails to expand

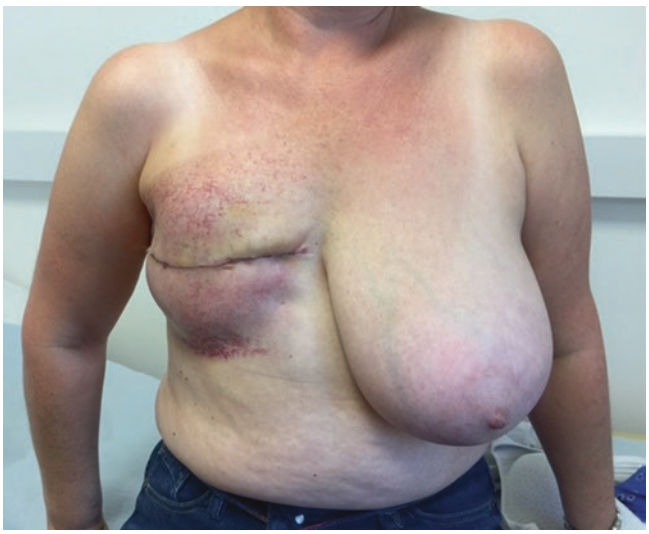

Fig. 35.10 Pt in Fig. 35.7. Upon exchange, an attempt to redefine the IMF by advancing abdominal wall tissue is only partially successful. The patient went on to have symmetrising reduction, however, and no longer needs an external prosthesis

  - At the time of expander placement and again at the time of exchange for an implant, the irradiated skin and fascia can be relaxed through multiple radial and horizontal 'capsulotomy' incisions, carried out from within the cavity (stopping before the dermis is reached). It is also safe to perform multiple capsulotomies following ADM-based procedures, as the dermal substitute should be fully incorporated into the surrounding tissues by the time of exchange.

- Because of resistance of irradiated chest wall tissues to expansion, clear definition and firm fixation of the IMF is crucial. This can be achieved in a number of ways.
  - At the time of expander placement, an ADM can be used to define the IMF, bearing in mind that the use of an ADM in this setting is associated with higher rates of infection and loss of both ADM and implant. The ADM will not expand itself and so in addition to fixation to the anterior rectus sheath and serratus fascia, it must be sutured to the lateral border of the pectoralis muscle with interrupted sutures; it may help to further shape it with radial incisions to encourage future expansion. Similarly, careful fixation of the ADM inferiorly prevents the expander from migrating downwards. This will not guarantee a well-defined IMF particularly in larger patients, or in those who have had an incomplete mastectomy leading to thick flaps.
  - At the time of replacement of an expander with a permanent implant, the IMF may require further definition. This can be achieved from within the implant pocket, and, here, the author's preference is to use several heavy PDS sutures to anchor the IMF from the deep dermis to the chest wall along a pre-drawn line corresponding to the level of the intended IMF. Forming the new IMF is facilitated by advancing the abdominal wall as described above, particularly when the soft tissues over the lower pole are tight and attenuated.
  - An alternative approach is to redefine the IMF by entering the implant cavity via an incision placed along the line of the planned IMF. A crescent of skin is then de-epithelialised, such that the full-thickness 'access' incision is in its centre. The upper part of this de-epithelialised crescent is then tacked to the chest wall with heavy interrupted PDS sutures. The abdominal wall is advanced to the same level and in a similar way is also tacked to the chest wall with heavy PDS sutures, since it will now be under some tension. The exact mark-up of the de-epithelialised section will vary and depend on skin laxity, thickness of the abdominal wall and degree of projection to be achieved after expansion. Contrary to traditional teaching, the authors have not found that fashioning these parallel 'tramline' incisions carries a risk of ischaemia, as long as the original mastectomy incision is mature, preceding the inframammary fold incision by at least 6 months.
- Patients with a with previously irradiated chest wall should be warned that, more than any other patient undergoing breast reconstruction, the likelihood of requiring further unplanned operations (for any reason, including repeated adjustments, implant exchange and revisional surgery) is very high [12].

35.6 Summary

Two-stage implant-based breast reconstruction is an attractive, relatively uncomplicated option, particularly for those women who are not suitable for other forms of breast reconstruction. Its appeal lies in its simplicity, low morbidity, short hospital stay and rapid recovery. Women should be informed that these shorter-term gains need to be considered carefully alongside the more favourable longer-term aesthetic outcomes associated with autologous tissue-based techniques. They should also understand that although the initial surgical episode is usually uncomplicated, they are likely to require long-term maintenance, with revision and possibly conversion of their reconstruction in the years which lie ahead.

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The Pedicled TRAM Flap in Breast Reconstruction

36

Glyn Jones

36.1 Introduction

Breast reconstruction using autologous techniques [1] have undergone considerable change in recent years. Earlier implant and expander-implant reconstructions achieved mound creation, but a natural ptotic breast shape remained an elusive goal. Recent advances in implant shape and texture technology coupled with the use of acellular dermal matrices have done much to dramatically improve implant-based outcomes in reconstruction, constituting over 80% of all breast reconstructions performed in the United States at this time. Some surgeons find the attendant complication rates and need for long-term maintenance troubling. Distortion and capsular contracture did little to encourage reconstructive surgeons and may well have contributed significantly to Veronesi's successful focus on breast conservation therapy. While capsular contracture is no longer the major problem it used to be, implant-based reconstruction definitely requires ongoing maintenance in the long term. The advent of autologous techniques with the latissimus dorsi and then transverse rectus abdominis myocutaneous (TRAM) flap and its derivatives for breast reconstruction revolutionized breast reconstruction, enabling surgeons to create a breast that is soft, warm, and well integrated into a patient's psyche. The latissimus flap often requires an additional implant, but TRAM flap techniques enabled us to create a truly autologous breast reconstruction without the need for long-term maintenance or adverse events. The popularity of skin-sparing mastectomy (SSM) and nipple-sparing mastectomy (NSM) has made further landmark advances in breast reconstruction, attaining the goal of a natural, almost scarless reconstructed breast. These oncologically safe procedures do not compromise mastectomy outcomes [2–4]. Combined with TRAM flap reconstruction whether pedicled or free, the technique offers potential for increasing patient acceptance of mastectomy as an alternative to breast conservation therapy (BCT). In our practice, 95% of patients undergoing mastectomy select immediate reconstruction with skin- or nipple-sparing mastectomy, while a minority present for delayed reconstruction.

36.2 History of the Pedicled TRAM Flap

Millard described the use of a tubed lower abdominal pedicled flap in reconstructing the radical mastectomy defect in 1976 [5]. The flap was transferred onto the chest via the forearm, using a waltzing technique, achieving a successful autologous tissue reconstruction for the time. In 1979, Robbins used a vertical rectus abdominis flap for breast reconstruction [6]. Independently, Drever, Dinner, and Sakai all refined variations on the use of vertical rectus abdominis myocutaneous flaps for breast reconstruction [7–10]. Hartrampf and Scheflan took the bold step of changing the skin island orientation to a transverse one across the mid-abdomen, making a more sizeable volume of tissue available for breast reconstruction with a cosmetically desirable donor site [11–14]. Scheflan confirmed the dominant deep inferior epigastric arterial supply to the lower abdominal skin and fat. Blood supply was most reliable directly over the designated rectus muscle from which perforators were most abundant, while the periphery of the flap relied on the capture of successive angiosomes through the subdermal plexus. Milloy had documented the blood supply of the rectus muscles in 1960, and these findings together with Scheflan's dissections found their culmination in the lead oxide injection studies of Taylor, Moon, and Palmer. Their publication of the angiosome concept was an extension of Michel Salmon's anatomic studies [15–17]. From these beginnings, the TRAM flap became the gold standard procedure for breast reconstruction and remains in widespread use today. In the interim, free flap options have arisen as refinements of the original pedicled technique, including the free TRAM (FTRAM), the muscle-sparing free TRAM, and the deep inferior epigastric

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artery perforator (DIEP) flaps, as well as the superficial inferior epigastric perforator (SIEA) flap. While the younger generation of plastic surgeons is rarely trained to perform pedicled TRAM flaps, they are still widely performed in the United States as well as in Europe at this time. Part of this relates to the dramatically shorter operating times, easier technical procedure, and lack of potential total flap loss in the pedicled flaps. Physician reimbursement has certainly impacted the nature of autologous reconstruction in some areas where perforator flaps are consistently rewarded with higher payment schedules.

36.3 The Vascular Anatomy of the Pedicled TRAM Flap

The skin and fat of the lower abdomen and periumbilical area are supplied by perforators arising from five major sources:

- Superior epigastric vessels arising from the termination of the internal mammary vessels
- Deep inferior epigastric vessels
- Superficial inferior epigastric vessels
- Intercostal segmental vessels
- Terminal branches of the superficial and deep circumflex iliac vessels

Of these, only the superior epigastric vessels are utilized when raising a pedicled TRAM flap. The predominant blood supply of the lower abdominal tissues is, however, unquestionable from the deep inferior epigastric system [14, 16, 17]. The vessels from both epigastric systems penetrate the rectus muscles on their deep surfaces and travel as single or duplicated vessels up and down the flap to anastomose in the periumbilical region through a system of choke vessels described by Taylor [17–19].

Three vascular patterns have been identified within the rectus muscles:

Type I (29%) had a single superior and inferior arterial supply.
Type II (57%) had a double-branched system from each source artery.
Type III (14%) had a triple-branched system from each vessel.

Bilateral vascular symmetry was noted in only 2% of patients.

Moon and Taylor proposed three variations in skin island design: the upper, mid-abdominal, and lower abdominal flaps. Vessels injections studies suggested increasing paucity of inflow the lower the skin paddle was placed on the abdominal wall in the case of a pedicled flap. This finding was corroborated by Harris [20] and has led to most surgeons designing the flap from just above the umbilicus to above the pubic crease and not below it.

Macroscopic communication between the two systems is present in only 40% of cases, while 60% of patients have choke vessels of microscopic caliber [16, 21]. These choke systems allow for reversal of flow to open up between the two systems to provide increased blood flow to the tissues. The superior vessels pass into the muscle from the deep aspect of the costal margin and run inferiorly. The deep inferior epigastric supply enters the posterolateral aspect of the muscle below the arcuate line and passes up to anastomose with the superior vessels in the periumbilical area. It provides significantly more circulation to the flap and is accompanied by two large venae comitantes which drain into the iliac circulation [14, 19]. These venae comitantes are usually larger than the superior veins, which partially explains the improved venous drainage associated with the free TRAM. The most dominant venous outflow is often supplied by the superficial inferior epigastric vein, the basis of the SIEA flap. This fact can lead to venous congestion in free flaps requiring decompression through the superficial inferior epigastric vein (SIEV). The periumbilical anastomosis has a bidirectional venous outflow confirmed by Taylor. Following elevation of a pedicled TRAM flap, distal venous flow has to reverse, following the drainage pattern of the superior veins. In order to achieve this, the venous flow pressure has to overcome the venous valves within the choke system described by Taylor [16, 17]. Arterial perforators arise from both systems and run in two roughly parallel lines on either side of the linea alba. The lateral row lies 2–3 cm within the lateral border of the rectus sheath, while the medial row lies 1–2 cm from the linea alba [17]. These vessels vary significantly in both size and number; their caliber may be miniscule to several millimeters in diameter.

The anterior rectus sheath is densely adherent to the muscle at the tendinous inscriptions. During flap elevation, a gently tapering cuff of this fascia is left on the muscle with its apex toward the costal margin helping to maintain the integrity of the muscle, thereby reducing the risk of injury to the pedicle. It also aids in reducing tension during closure [22]. A muscle-sparing technique can be used to leave a strip of muscle laterally and/or medially to assist in maintaining abdominal wall strength, but the rationale for this is flawed as it is poorly vascularized (if at all) and probably just contributes some fibrous scar to the long-term abdominal closure. The intercostal nerves and vessels penetrate the posterior aspect of the rectus muscle at the junction of the mid and lateral thirds of the muscle and not in the lateral third. Any lateral segment is probably devoid of neurovascular input [23, 24]. Harris demonstrated an 80% reduction in intraoperative blood flow when clamping the medial and

lateral thirds of the rectus muscle to simulate muscle-sparing harvest [20]. Consequently, there seems little value to incorporating muscle-sparing surgery into TRAM flap harvest.

36.4 Vascular Zones in TRAM Flap Blood Supply

Two major vascular classifications exist for TRAM flap blood supply. The best known and earliest description was that of Hartrampf (Fig. 36.1) who divided the supply into four zones:

Zone I overlying the muscle pedicle
Zone II lying across the midline, immediately adjacent to zone I
Zone III lying lateral to zone I on the ipsilateral side
Zone IV lying lateral to zone II on the contralateral side from the pedicle

Historically, zone I is the most reliable portion of the flap. This was followed by the medial portion of zone III. The end of zone III becomes increasingly unreliable as one moves toward the tip of the flap and it is wise to discard it in most patients. The medial portion of zone II is also usually reliable, but the lateral part is less predictable followed by zone IV which should be discarded routinely. Taylor documented the anatomic theory behind this approach in his paper on the angiosome concept [16]. It is his belief that a single adjacent vascular territory could be captured relatively reliably, but more than one angiosome capture becomes increasingly unpredictable, particularly once the midline is crossed. These observations led Taylor to popularize the concept of TRAM flap delay in an effort to bolster the blood flow to the flap prior to elevation.

Ninkovich and Holm [25] performed dynamic indocyanine green perfusion imaging of DIEP flap blood supply in vivo. They reached the conclusion that while zone I remains the most reliably perfused portion of the flap, any flow across the midline is more precarious than ipsilateral flow. The Ninkovich classification proposes that Hartrampf's ipsilateral zone III should be renamed zone II, while Hartrampf's zone II should be renamed zone III with a less reliable flow due to its cross midline location (Fig. 36.2). One of their DIEP flaps is shown in Fig.

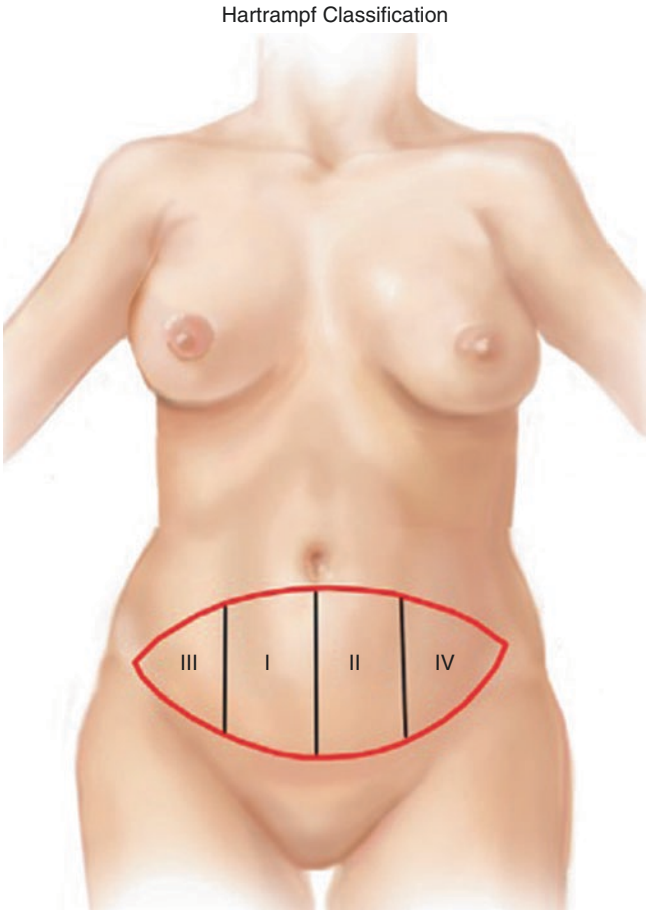


Fig. 36.1 Hartrampf's classification of TRAM flap blood supply zones

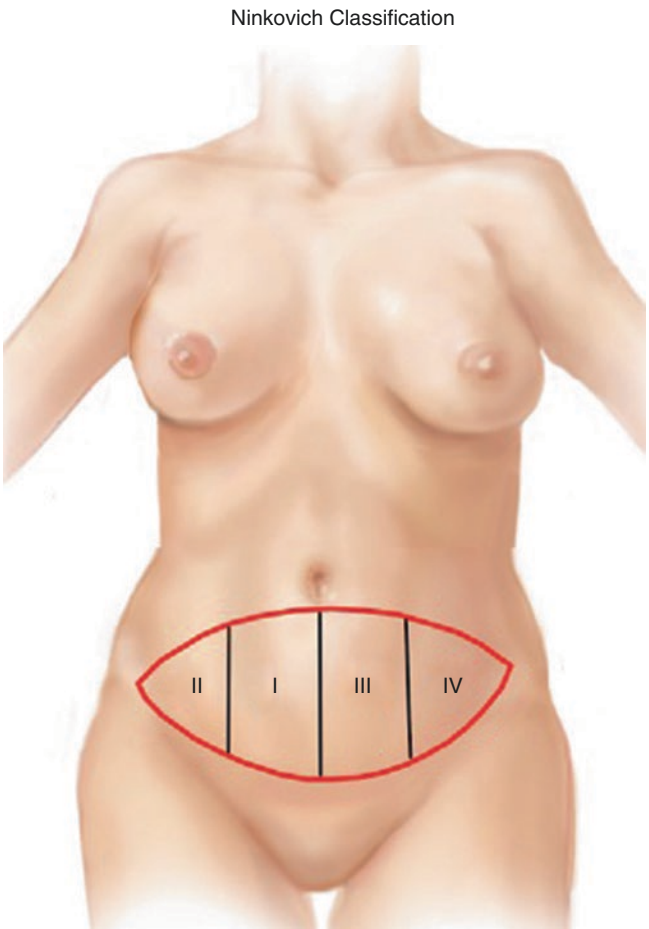


Fig. 36.2 Ninkovic's classification of TRAM/DIEP flap blood supply zones

(Fig. 36.2) clearly illustrating this phenomenon with all of the cross midline tissue showing signs of ischemia, while the ipsilateral tissue remains well perfused. These findings are borne out by both reperfusion times and the rate of flow in each zone. There is an increasing trend among both pedicled and free flap surgeons to rely more on the ipsilateral side than any cross midline tissue in an effort to reduce flap necrosis.

36.5 The Anatomic and Physiologic Basis of TRAM Flap Vascular Delay

Vascular delay is a historic concept, the efficacy of which was documented during the era of tube pedicled flaps for general reconstruction [26]. Moon and Taylor recommended surgical delay of the TRAM flap 1 week prior to definitive elevation. The procedure focused on ligation of the superficial and deep inferior epigastric systems in an outpatient setting. Although timed for 1 week prior to flap elevation and breast reconstruction, Taylor believes the delay phenomenon reaches its functional peak at 72 h after surgery rather than the classic 10-day window suggested in past literature [26]. The procedure is effective but adds another step to the operation with added costs incurred. One creative approach used by Rezai is to perform a contralateral breast reduction at the time of vascular delay, coming back several days later to transfer the vascular-delayed TRAM flap to the fresh mastectomy site. This maximizes operating room time utilization. Codner demonstrated a statistically significant rise in vascular inflow to the pedicle after delay with improved perfusion pressures in the vascular-delayed cases [27]. This was corroborated by Restifo and Ribuffo [28, 29]. Restifo demonstrated a flow in the superior epigastric vessels similar to that of the inferior system once delay had been performed. He was also able to demonstrate no additional benefit to waiting longer than 1 week after delay prior to formal flap elevation. Ribuffo used color Doppler studies to demonstrate increased caliber of, and flow within, the superior epigastric system after vascular delay [29]. The indications for delay can be summarized as follows:

- An alternative for plastic surgeons not comfortable with microsurgical reconstruction
- Useful for the higher-risk patient—obesity, smoking, and prior radiation to the proposed TRAM flap pedicle

36.6 Abdominal Anatomy and the Use of Pedicled TRAM Flaps

Competent rectus sheath closure is an essential element to success with any TRAM flap procedure, be it pedicled or free [22, 30, 31]. Laterally the rectus sheath consists of two fascial components derived from the external and internal oblique muscles. These blend into a confluent anterior sheet which fuses at the linea alba with the contralateral sheath. It is imperative that *both* lateral components be incorporated into the fascial closure when closing the donor defect if hernias or bulges are to be prevented [22, 32]. Nerve supply to the muscle is segmental and must be divided when raising the flap. It is essential to denervate the eighth intercostal nerve at the costal margin as this maneuver causes the muscle to atrophy so as to prevent muscle bulging at the costal margin tunnel when the patient sits up.

36.6.1 The Case for Delayed or Delayed-Immediate Reconstruction

Immediate breast reconstruction is the most favorable context in which to perform breast reconstruction as it offers the benefit of retaining the natural breast skin envelope's shape and consistency. It also retains the natural location and composition of the inframammary fold, assuming that the breast surgeon has not violated this critical structure. This leaves behind the patient's natural skin brassiere together with a defined inframammary fold [2] to help mold the newly reconstructed breast. While this is most valuable in immediate reconstruction, it does offer maintenance of the inframammary crease for delayed reconstruction. The original breast shape may be more readily matched as a consequence [33].

Traditional mastectomies leave a large skin defect making access to the chest wall, axilla, and TRAM flap tunnel communicating with the abdominal dissection very simple. In skin-sparing mastectomy, the excision usually incorporates a peri-areolar biopsy if this has been performed and axillary dissection is either done through the nipple-areola complex (NAC) wound or through a separate axillary incision [3]. The closer an excised skin biopsy site is to the excised nipple-areola disk, the greater is the risk of skin bridge necrosis. Skin incisions for the procedure have been suggested by Carlson, Toth, and Skoll, all of whom highlight the risks of the Wise pattern approach for ptotic patients [3, 34, 35]. Skin flaps must be handled gently in order to minimize the risks of skin necrosis. Every attempt should be made to preserve the inframammary fold as Carlson has shown that this does not

compromise the oncologic safety of the procedure and it greatly enhances the ultimate appearance of the reconstruction [36] . Although skin-sparing mastectomy may be used without immediate reconstruction, the retention of the additional breast skin does little to ease the reconstructive surgeon's task when delayed reconstruction is finally performed. This is particularly true of radiated patients. Data published by Kronowitz [37] has supported the concept of immediate-delayed reconstruction. In this process, mastectomy flaps may be held out to size by an immediately placed expander which can be inflated and then deflated during radiation if reconstruction is to be delayed, followed by re-expansion and either implant or free flap insertion. This approach has been given the label of "immediate-delayed" reconstruction but has a reputation for 35% complication rates from seromas and infection. Andree has more recently proposed the IDEAL concept of delayed reconstruction in which an implant and not an expander is placed and used as a spacer until radiotherapy has been completed, followed by early DIEP flap substitution. It does allow the surgeon to take advantage of nipple- or skin-sparing mastectomy in the face of delayed reconstruction.

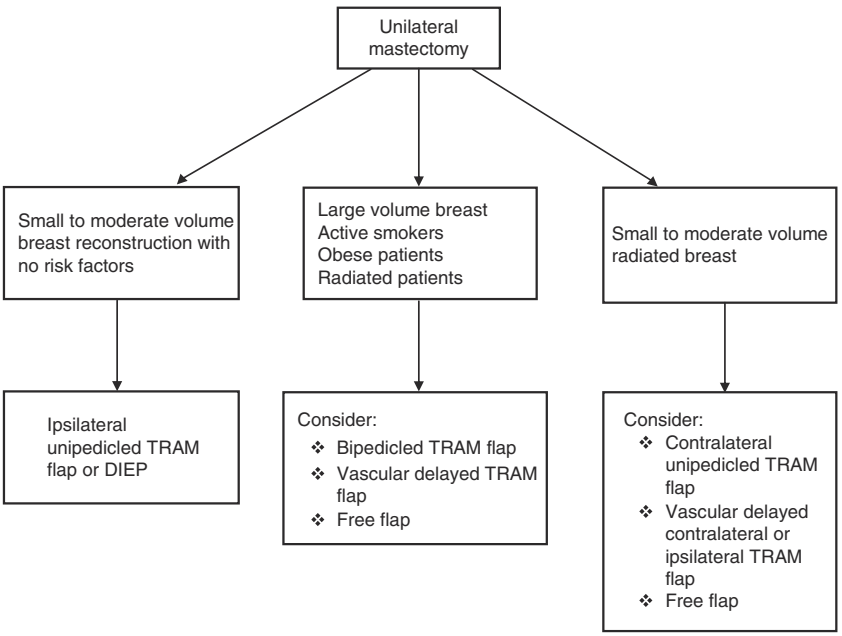
36.7 Patient Selection for TRAM Flap Breast Reconstruction

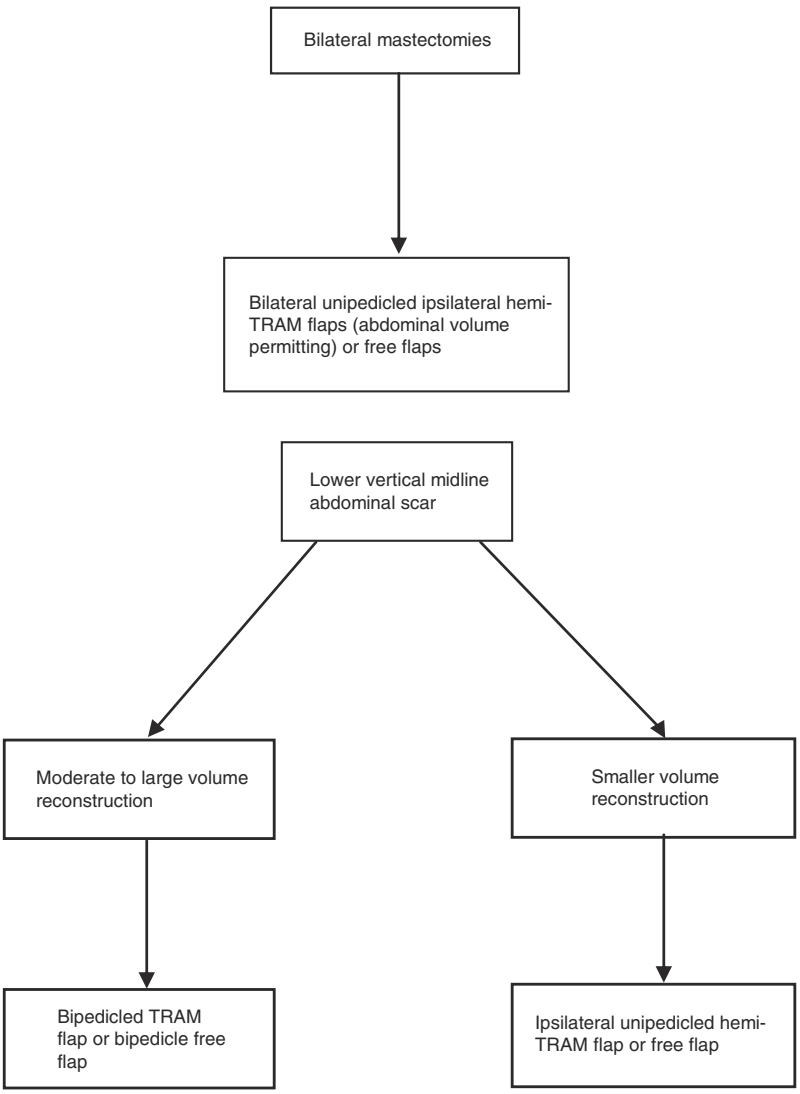
The first prerequisite for this procedure is a patient healthy enough to undergo a 2–3-h operation, a 3–5-day hospital stay, and a 4–8-week recovery period before the patient begins to feel that life is returning to some degree of normality. The second major requirement is an available donor site. The patient should have a thorough history taken including an evaluation of comorbidities such as gastroesophageal reflux disease (GERD), irritable bowel syndrome, lumbar spine problems, smoking history, and cardiovascular risk factors. We did not find diabetes mellitus to be a risk factor in TRAM flap usage [32] although Hartrampf has assigned it a significant value [11]. Collagen vascular disease is potentially problematic although we have performed the procedure safely in patients with systemic lupus erythematosus and mild rheumatoid arthritis. Scleroderma would present more of a risk if anterior chest tightness were present as this could compromise abdominal skin closure. Considerable caution should be exercised in deciding to operate on a patient with Ehlers-Danlos syndrome. A history of prior abdominoplasty or abdominal liposuction represents relative contraindications to the procedure in theory (although we have successfully performed the procedure in a patient with complete abdominal wall undermining 20 years previously as well as in patients with conservative liposuction). Preoperative CT or MR angiography is not particularly helpful in pedicled TRAM flap planning, but delineation of perforator location is helpful in planning perforator flaps. Intraoperative indocyanine green perfusion assessment is more helpful in this author's experience. Clinical examination should be performed noting body habitus and weight. The abdomen should be examined for old scars, particularly cholecystectomy scars or vertical midline incisions [38]. Pfannenstiel incisions are not a risk factor. Laparoscopic incisions are rarely a problem, but port sites may injure the vessels within the rectus muscle in the upper abdomen, and Doppler evaluation is probably prudent during surgery. It is unwise to operate within 6 weeks after laparoscopic surgery. A final factor in flap selection is that of the patient's occupation and lifestyle. Very active, young individuals are better served by a DIEP or SIEA flap, and patients engaged in musical careers occasionally express concerns about the impact of muscle loss on their ability to sing. This does not appear to be a significant issue in practice.

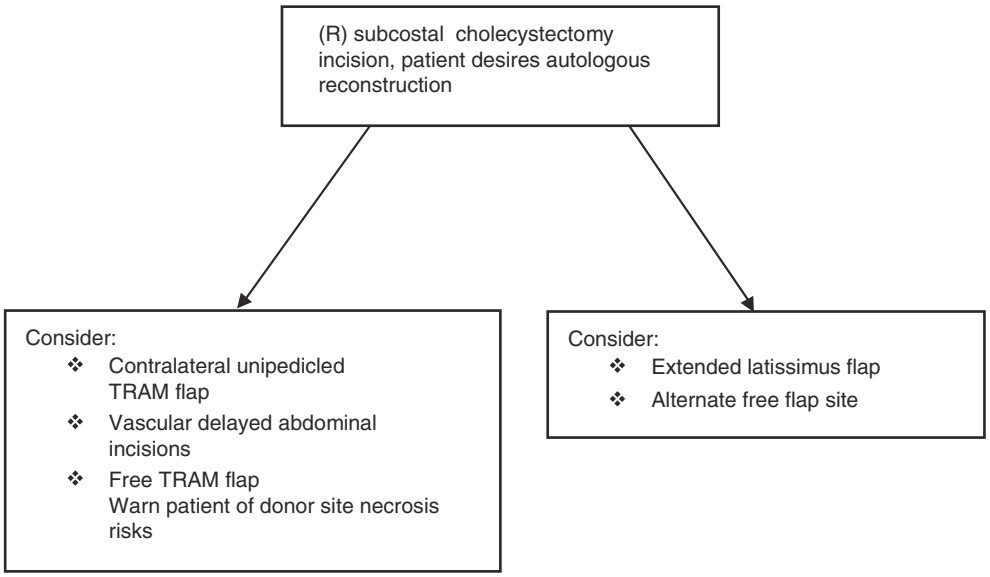
Hartrampf attempted to assign risk scores to patients in order to determine their eligibility for TRAM flap reconstruction [11]. Risk factors included smoking, obesity, psychological instability, autoimmune disease and diabetes mellitus, severe systemic disease, and surgeon inexperience. Using this rating system, a patient with two risk factors or a score of <5 represented a borderline risk, while patients with three or more risk factors or a score of >5 were considered a poor candidate for surgery [11]. In our own series, diabetes did not correlate well with complications, but obesity, smoking, abdominal scars, and prior radiation therapy did [31, 32]. The following algorithm is an attempt at simplifying flap choices for patients with differing risk factors as well as including the surgeon's preference and level of comfort with microsurgery.

36.7.1 TRAM Flap Selection Algorithms

1. Small- to moderate-volume breast reconstruction with no risk factors—ipsilateral unipedicled TRAM flap or DIEP flap
2. Large-volume breast, active smokers, obese patients, and radiated patients—bipedicled TRAM flap, vascular-delayed TRAM flap, and free flap (excluding DIEP)
3. Small- to moderate-volume radiated breast—contralateral unipedicled TRAM flap (or ipsilateral TRAM flap if radiation injury appears mild) or free flap


Unilateral mastectomy
Small to moderate volume breast reconstruction with no risk factors
Large volume breast
Active smokers
Obese patients
Radiated patients
Small to moderate volume radiated breast
Ipsilateral unipedicled TRAM flap or DIEP
Consider:
Bipedicled TRAM flap
Vascular delayed TRAM flap
Free flap
Consider:
Contralateral unipedicled TRAM flap
Vascular delayed contralateral or ipsilateral TRAM flap
Free flap


Bilateral mastectomies
Bilateral unipedicled ipsilateral hemi-TRAM flaps (abdominal volume permitting) or free flaps
Lower vertical midline abdominal scar
Moderate to large volume reconstruction
Smaller volume reconstruction
Bipedicled TRAM flap or bipedicle free flap
Ipsilateral unipedicled hemi-TRAM flap or free flap



36.8 Anesthetic Requirements

Patients are kept warm and well hydrated to provide robust circulation [11]. Urine output should be high throughout the procedure. Nitrous oxide administration can cause small bowel distention resulting in potential difficulties with abdominal wall closure; nitrous oxide inhalation is not used at all in our practice. Intraoperative body-warming blankets are used routinely as are leg compression stockings. We use prophylactic heparin therapy or low molecular weight enoxaparin as these drugs do not appear to increase the risk of hematomas. Intravenous ketorolac for postoperative pain has not been shown to increase hematoma rates [39, 40]. The reported incidence of deep venous thrombosis complicated by pulmonary embolism in our series was just under 0.006%, while the incidence of fatal pulmonary embolism is approximately 0.1% [31, 32].

36.9 Unipedicled Operative Procedure

The upper abdominal incision is made first and the upper abdominal skin flap is elevated over the costal margins laterally and to the xiphoid centrally. The patient is flexed to assess the adequacy of closure to the inferior incision line. The inferior incision may need to be elevated slightly to allow for a less tense suture line in patients with a long narrow torso. Tight closure can seriously compromise blood flow to the skin edges causing skin necrosis. Obese patients are particularly at risk. Pfannenstiel incisions are routinely ignored. The distal incision is then made, and TRAM flap is elevated from lateral to medial identifying the lateral row of perforators and the lateral border of the rectus abdominis muscle. The decision as to which side to base the flap depends upon abdominal anatomy and surgeon preference. In the unscarred abdomen, either side may be used and I prefer the ipsilateral pedicle (Figs. 36.3 and 36.4a, b). Ipsilateral

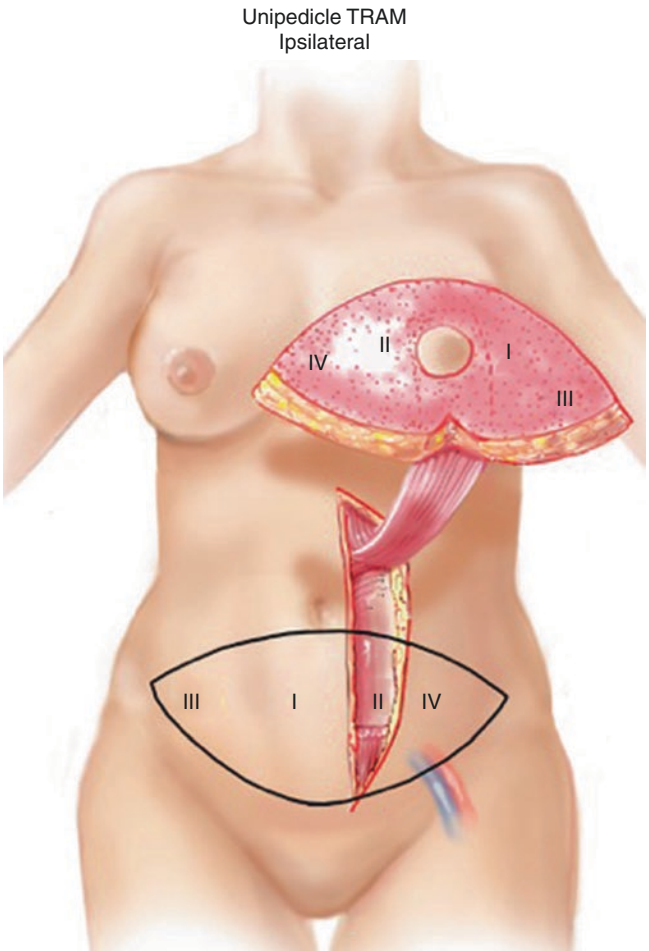


Fig. 36.3 Ipsilateral unipedicled TRAM flap with 180° flap rotation

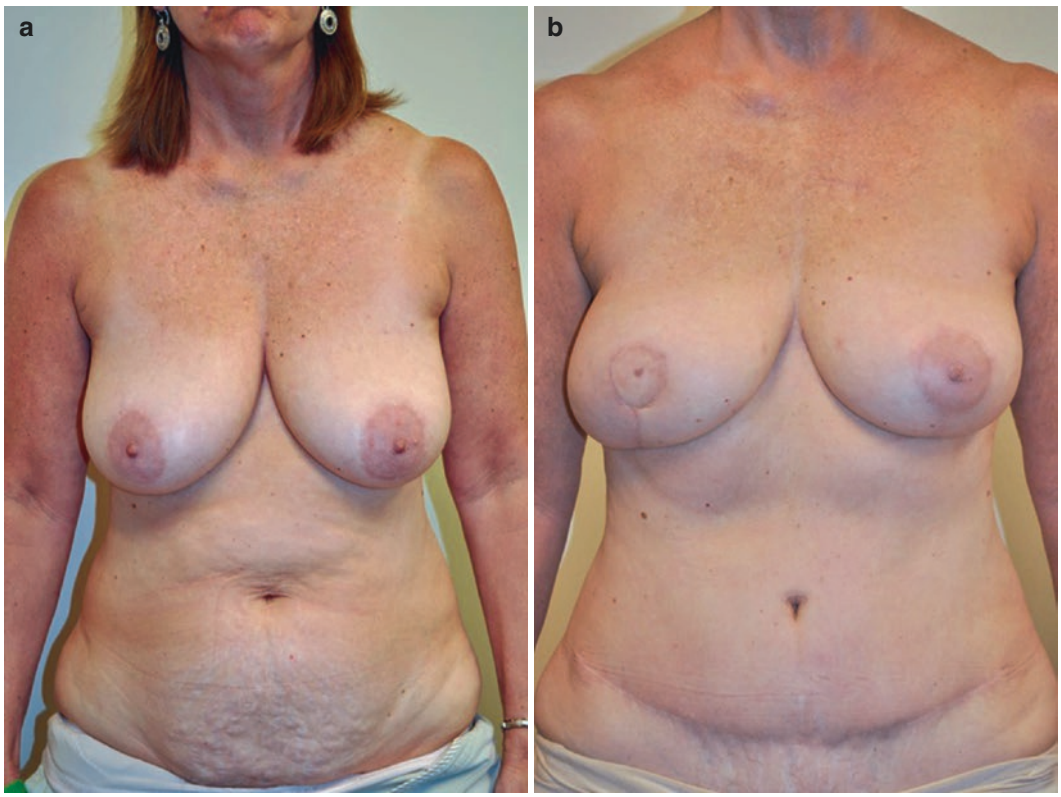


Fig. 36.4 (**a**) Preoperative views of patient with DCIS of the left breast. (**b**) Postoperative views after (L) ipsilateral TRAM flap reconstruction

transfer reduces initial intermammary bulging, and the definition of the ipsilateral inframammary crease tends to be excellent. Pedicle tension is reduced and flap positioning is easier. Venous drainage of the flap appears better with ipsilateral transfer [41]. The contralateral pedicle (Fig. 36.5) tends to create more blunting of the medial inframammary crease and limits the ease of flap positioning laterally.

Radiation to the affected breast necessitates either a contralateral unipedicled flap (with or without surgical delay) or preferably a bipedicled or free TRAM flap. While the ipsilateral radiated pedicle can be used in many patients, it may be unreliable and I always use Doppler to assess the ipsilateral pedicle signal and compare it to the non-radiated side. We have clearly shown a higher fat necrosis rate in patients with preoperative radiation to the internal mammary supply [42]. A contralateral pedicle is useful in such cases but tends to cause some degree of blunting of the medial inframammary fold and softens the depth of the intermammary cleavage, although careful denervation of the pedicle may ameliorate this problem**.** A surgical delay performed 5–14 days previously improves TRAM flap blood supply and may be considered for a pedicled procedure if a large breast is to be fashioned [26, 27, 43, 44]. If vascular delay is performed, it should include an incision right across the lower inferior end of the TRAM flap with elevation of the flap tips as described by Taylor [26, 45] and not just two small groin incisions to give access for vascular

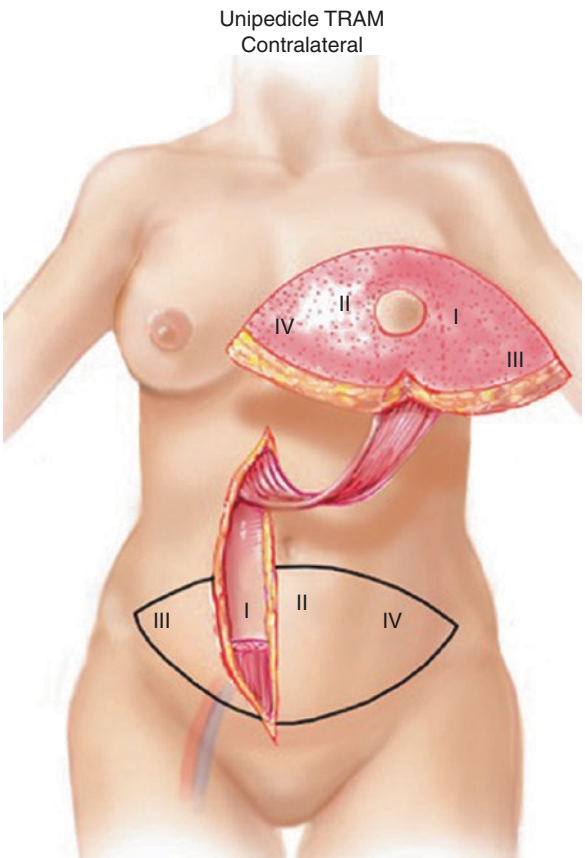


Fig. 36.5 Contralateral unipedicled TRAM flap with 180° flap rotation

division. The rectus fascia is incised as a long ellipse to facilitate closure and maintenance of muscle integrity at the inscriptions and is freed from the underlying rectus muscle. Care must be taken not to penetrate the muscle while separating the tendinous inscriptions. The muscle can be elevated in its entirety or using a muscle-sparing technique (Fig. 36.6). Muscle sparing involves identifying the intramuscular course of the superior epigastric vessels with a Doppler probe and then leaving a lateral strip of muscle some 2 cm in diameter. Theoretically, this leaves muscle innervated and vascularized by the intercostal vessels and nerves for further abdominal wall competence postoperatively. In practice, however, the intercostal supply penetrates the rectus muscle in its middle third, thereby leaving no innervation and probably little, if any, blood supply to the lateral muscle strip. A medial strip of muscle may also be left but its functional value is also questionable. As noted earlier, Harris demonstrated an 80% reduction in pedicled blood flow by clamping the medial and lateral thirds of the rectus muscle intraoperatively [20]. Suominen's data on the diminishing size and strength of residual upper rectus muscle left after free TRAM flap harvest calls into question the validity of performing muscle-sparing procedures [24]. The rectus muscle is divided distally, and the deep inferior epigastric vessels are ligated with LIGACLIP®. These vessels should be dissected out with the flap just in case they are needed for conversion to a free flap in the event of vascular compromise of a pedicled flap.

Flap elevation is based on the superior epigastric supply. Care should be taken to divide the eighth intercostal nerve as it enters the muscle near the costal margin. This causes muscle atrophy, reducing epigastric bulk in the long term. A wide subcutaneous tunnel is made between the abdominal dissection and the mastectomy site allowing passage of the pedicle without compression. When using a contralateral pedicle, it is tunneled adjacent to the medial border of the normal breast. Ipsilateral flaps are passed straight up through the inframammary fold of the mastectomy site. If venous congestion occurs, repositioning may be helpful. Additionally, one may remove the LIGACLIP on the deep inferior epigastric vein stump and allow it to bleed for several minutes for venous decompression.

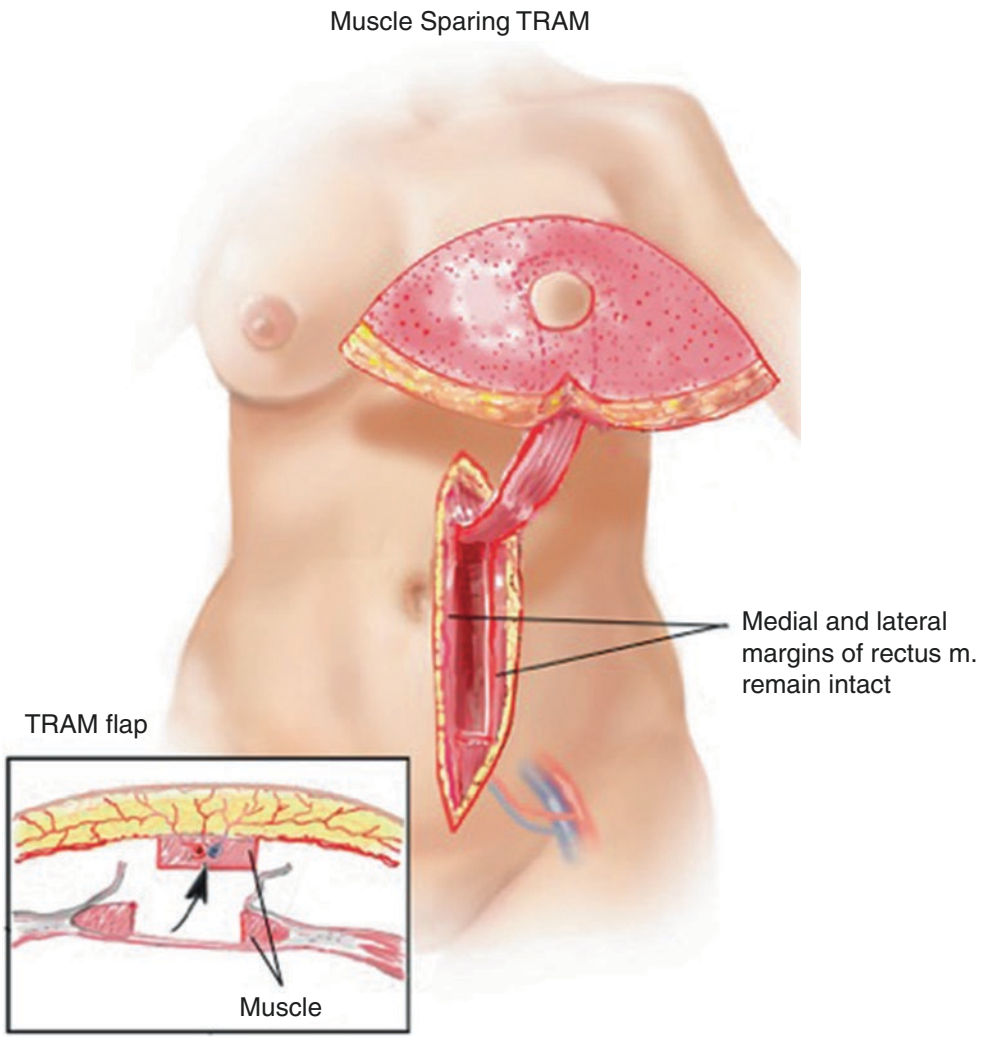


Fig. 36.6 Muscle-sparing unipedicled TRAM flap

Alternatively, Hartrampf's "mechanical leech" drainage system may be inserted into the deep inferior vascular system to aid in venous decompression [46].This involves inserting a pediatric feeding tube or venous cannula into the deep inferior epigastric vein and using this as a decompression valve which can be opened periodically to bleed the flap of congested, poorly oxygenated venous blood under pressure. The catheter can be flushed with dilute heparin solution to maintain its patency over a period of 2–3 days as needed.

Abdominal closure should be performed meticulously as poor closure dramatically increases the risks of hernia formation. It is essential to incorporate both the internal and external oblique aponeuroses into the sheath closure [47]. If fraying of the fascia occurs, it can be darned with a suture weave or covered with an onlay of AlloDerm®. Bucky and May have reported the routine incorporation of mesh into all TRAM flap abdominal closures with excellent success; one patient of 65 patients treated developed a mesh infection, and one patient developed hernia [48]. Once abdominal fascial closure has been securely closed, the upper abdominal skin flap is redraped over suction drains and closed. An umbilicoplasty is then performed. TRAM flap shaping follows and technical caveats are discussed later in this chapter. Careful attention should be paid to recreating the lateral inframammary fold with quilting sutures to prevent loss of definition at this site. This maneuver should be performed with the patient in the erect position to evaluate the effect of gravity on the final shape of the reconstruction.

36.10 Bipedicled TRAM Flap

The bipedicled TRAM flap is potentially indicated in:

1. Large-volume reconstruction
2. Patients with midline abdominal incisions
3. Smokers
4. Obesity
5. Patients with radiation injury to one pedicle

Most of the above represent indications for free TRAM flap transfer in many surgeons' hands. Bipedicled flaps are robust and probably have a better blood supply than free TRAM flaps due to the conversion of zones II and IV to additional zones I and III, respectively. They allow for more reliable survival of a greater proportion of the flap at the expense of greater abdominal donor site muscle loss. While this impacts the patient's abdominal strength in the short term, longer-term function appears eminently compatible with activities of daily living. Flap complications are less, and the procedure enables the non-microsurgeon to safely perform TRAM flap breast reconstruction in higher-risk patients [31].

Preoperative preparation and positioning are similar to those outlined for the unipedicled procedure. Initial flap elevation is identical in that both sides of the flap are dissected to the lateral perforators. Medial dissection differs in that a tunnel must be fashioned down the linea alba between the two pedicles (Fig. 36.7). This leaves a fascial strip on either side of the linea for fascial closure. As two pedicles have to pass up onto the chest wall, a more generous tunnel has to be fashioned causing more initial bulging about which patients should be informed. Once the flap is elevated, it is passed onto the chest taking care to prevent compression of the pedicles within the tunnel. I use a dou-

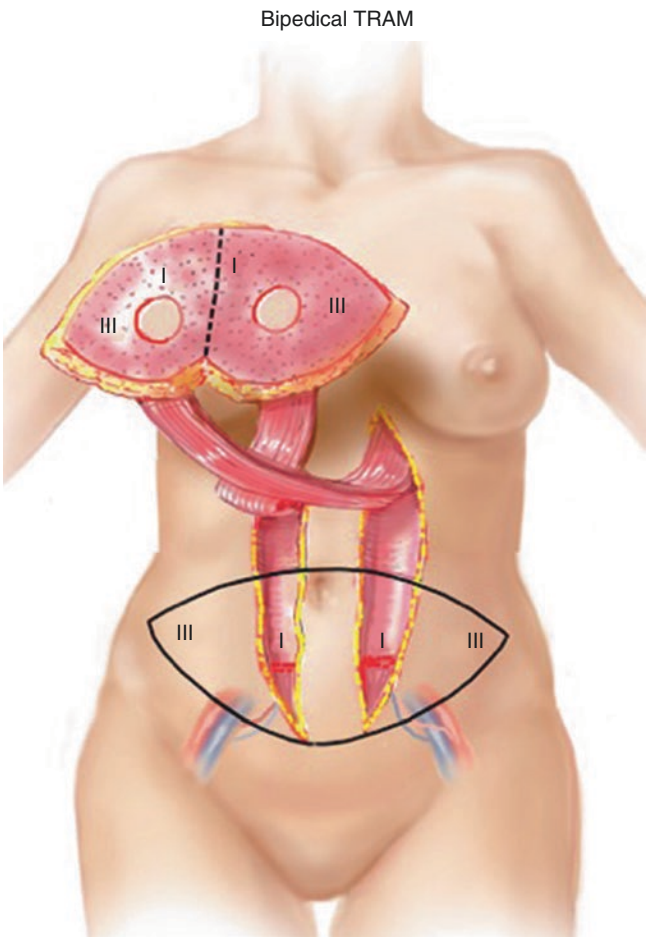


Fig. 36.7 Bipedicled TRAM flap transfer

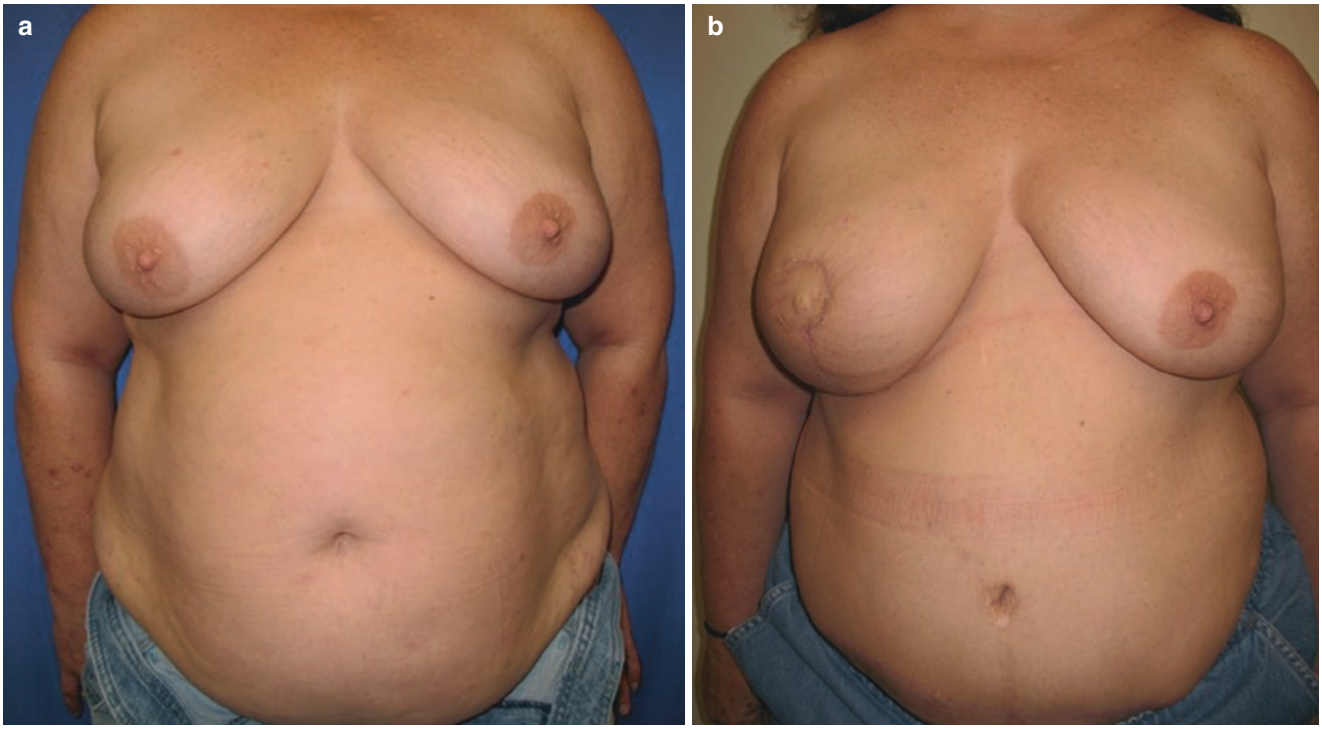


Fig. 36.8 (**a**) Preop view of obese patient with large-volume breast for TRAM flap reconstruction; (**b**) post-op result after bipedicled TRAM flap breast reconstruction

ble-layer mesh closure routinely in bipedicled flap procedures. The Prolene mesh is sutured from linea semilunaris to linea semilunaris using 0-Prolene. Abdominal wall strength is almost certainly more compromised when compared with the unipedicled procedure [49, 50], and it should be performed with caution in the younger patient. In the Emory review of bipedicled patient results, flap complications and abdominal wall complications were no worse than with unipedicled flaps, and flap blood supply was predictably better given the dual blood supply [31]. Our large experience with bilateral and bipedicled flaps has confirmed our initial experience with this procedure as being safe and reliable with remarkably few complications considering the higher-risk patients in whom it is performed. The abdominal strength objections voiced by some surgeons do not appear to be as significant as initially thought, and patients cope remarkably well with activities of daily living. While it is true that strength is diminished significantly initially, particularly with respect to patients' ability to perform sit-ups, abdominal wall function improves with time, and a remarkable number of patients achieve little or no negative impact on activities of daily living. Hernia rates are not significantly higher with this procedure when compared with unipedicled TRAM flaps. These issues will be discussed at greater length in the outcomes section. It is an excellent option for the non-microsurgeon who performs large numbers of breast reconstructions in higher-risk patients or those patients requiring large-volume reconstructions (Fig. 36.8b).

36.11 Bilateral Unipedicled TRAM Flap Breast Reconstruction

Bilateral reconstruction using two unipedicled TRAM flaps follows an identical operative sequence to that described for the bipedicled procedure, the exception being that the skin island is split down the midline during the initial dissection creating two flaps of equal size (Fig. 36.9a, b). The flaps are transposed to the chest wall through ipsilateral tunnels to prevent possible compression and kinking through a common central tunnel. Flap rotation on the chest wall is typically 90°. Abdominal closure is identical to that for the bipedicled TRAM flap.

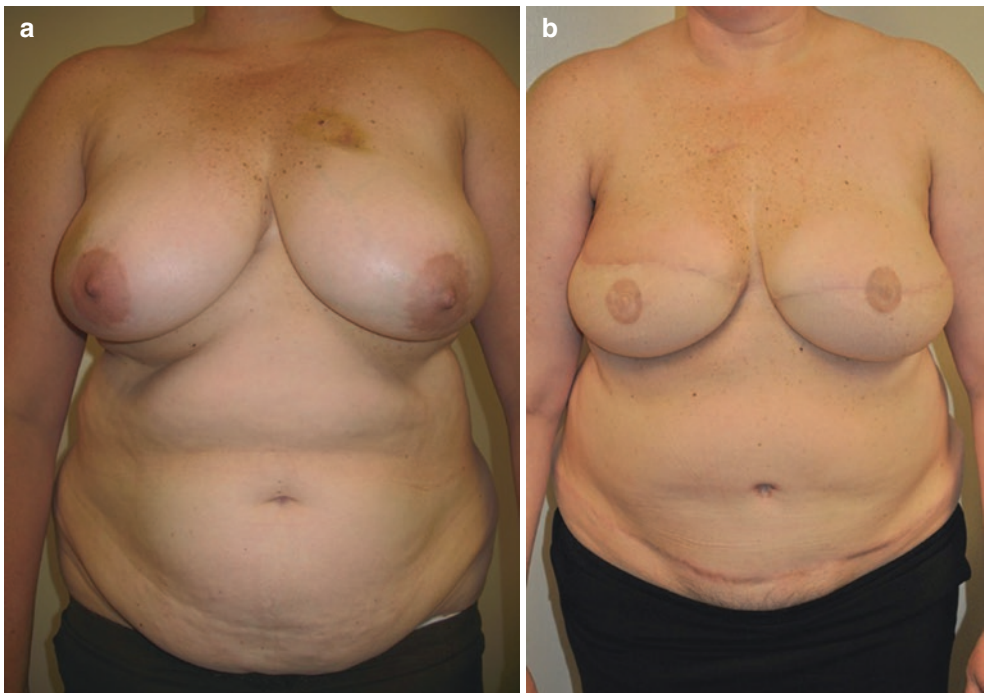


Fig. 36.9 (**a**) Preop view of obese patient requiring bilateral mastectomies and autologous reconstruction for breast cancer. (**b**) 1-year postoperative result after bilateral ipsilateral unipedicled TRAM flaps

36.12 Intraoperative Volume Assessment

During immediate reconstruction, the mastectomy specimen can be weighed off the surgical field. The problem becomes how to determine the volume of the TRAM flap available to achieve a match for the contralateral breast. Wagner devised a formula to calculate flap volume, $L \times W \times T \times 0.81 = V$ where L, W, and T represent the length, width, and thickness, respectively, of the TRAM flap [50]. Hudson has suggested the use of a simple hanging balance gas sterilized for intraoperative measurement of flap weight rather than volume [51]. Volumetric assessment by the hand is a simple but crude and somewhat inaccurate alternative.

36.13 Dealing with the Old Mastectomy Scar

The previous mastectomy scar, whether radiated or not, poses significant technical problems. If incised and used as the inset for the TRAM flap, its tight horizontal contraction tends to act as a band across the upper pole of the reconstruction, creating a linear groove. If this occurs, the scar should be excised completely, and a lateral modified Z-plasty should be created to soften the contour of the inset**.** The procedure is more of an oblique back-cut than a true Z-plasty, allowing a tongue of the TRAM flap skin island to angle up toward the axilla.

36.14 Flap Shaping and Positioning in Delayed TRAM Flap Reconstruction

The tip of zone I and all of zone IV should be discarded unless their blood supply appears unusually good. Many surgeons work primarily with ipsilateral tissue only in an effort to reduce fat necrosis. Flap orientation exerts a major influence on shape and symmetry. Secondary shaping is always feasible and often necessary [52], but time spent shaping the flap at the initial procedure is well spent, and it is possible to achieve excellent shape and symmetry at this first stage when adjustments are made most easily [33, 53–55]. The most common orientations used by the author are a transverse lie with a 180° rotation or an oblique orientation with a 120° or 80° rotation. Generally, it is preferable to place as much volume inferiorly in order to maximize projection and natural shape.

36.15 Donor Site Closure

Donor site closure is critical to the successful completion of TRAM flap breast reconstruction. A few words on donor closure are pertinent. Sheath closure should always incorporate both the internal and external oblique fascial layers to limit the risk of hernia formation. A deep layer of either running or interrupted #1Prolene suture followed by a second layer of running #1PDS suture is commonly used. Closure with double figure-of-8 #1Prolene interrupted sutures provides an extremely powerful closure with a built-in pulley-like

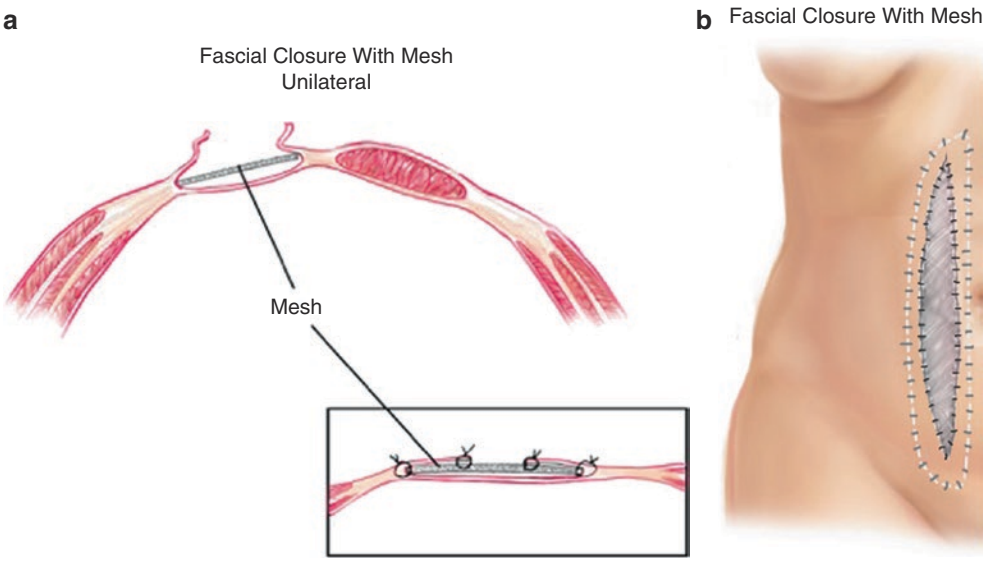


Fig. 36.10 (**a**) Unilateral mesh insertion within rectus sheath, (**b**) final anterior sheath overlay onto mesh inlay

mechanism to reduce fascial tearing as the fascia is closed. This is reinforced with a running #1PDS layer secondarily. Contralateral vertical sheath plication to centralize the umbilicus in unilateral pedicled flaps is unnecessary; if anything it simply raises intra-abdominal pressure unnecessarily and does little to move the umbilicus centrally. The skin pannus is defatted around the umbilicus inset, which the author prefers to reconstruct using the Avelar umbilicoplasty. Mesh should be used if there is extensive tearing of weak fascial components during closure or if tension seems high. If it is required, I use an inlay technique, suturing the mesh to the linea semilunaris internally within the sheath laterally and medially to the linea alba (Fig. 36.10a, b). The overlying anterior sheath leaflets are then sewn over the top of the mesh to cover about 50–60% of its surface area. Bucky describes excellent results with extensive inlay resulting in attractive abdominal contouring [48].

36.16 Timing of Nipple Reconstruction

It has been our policy to wait 6–8 weeks before performing nipple reconstruction. This allows the flap to settle under the influence of gravity, allowing nipple placement to be more accurately assessed. A C-V flap or modified skate flap is used for nipple reconstruction, creating a nipple some 50% longer than required, as atrophy will cause further slight loss of projection with time. In an effort to maintain nipple projection, I place the reconstructed nipple's free edge on a shelf of de-epithelialized adjacent TRAM flap skin to prevent the nipple from falling back into the donor site. Tattooing is usually performed 6–8 weeks later to minimize the effect of tattoo-induced atrophy of the nipple**.** Immediate nipple reconstruction has been advocated by some and is certainly more cost-effective than staged procedures [56]. The difficulty with using this approach is that settling of the TRAM flap may result in an incorrectly placed nipple reconstruction. The use of a traditional skate flap with surrounding skin graft unquestionably provides the best long-term projection in the author's opinion, but its requirement for a skin graft detracts from its value [57].

36.17 Secondary Shaping and Contralateral Breast Surgery for Symmetry

Secondary shaping is usually not necessary if careful attention to flap shaping and symmetry has been taken at the initial operation. Where possible, it is preferable to match the reconstruction to the unoperated contralateral breast unless this breast is in need of reduction or mastopexy at the patient's request. If secondary shaping is necessary, I prefer to perform it at the time of nipple reconstruction [52]. Careful contouring with 3–4 mm cannulae will help define blunted inframammary folds or lateral breast creases and effectively reduces minor contour defects produced by overfilling with excess flap bulk. Contralateral reduction, mastopexy, or augmentation will be necessary in some patients and can be performed either at the initial operation or subsequently at the time of nipple reconstruction.

36.18 Complications and Outcome Studies in TRAM Flap Reconstruction

The major complications of delayed TRAM flap reconstruction include scarring, skin and fat necrosis, flap loss, hernia formation, deep venous thrombosis, asymmetry, abdominal

tightness, and the psychosexual issues associated with breast reconstruction.

36.19 Skin and Fat Necrosis

Some degree of fat necrosis is common in any TRAM flap reconstruction whether free or pedicled. It should be less prevalent in free tissue transfers. The problem in assessing the available data is that authors differ in their estimate of "clinically significant" fat necrosis. Furthermore, many perforator flap surgeons only use ipsilateral tissue and discard any cross midline tissue. In our series at the Emory Clinic, we used a definition of 10% or more of the flap surface containing palpable firmness and included cross midline tissue in most of our reconstructions. This definition yielded a "significant fat necrosis" rate of 10.6% [32]. Risk factors associated with fat necrosis included prior radiation ($p < 0.001$), abdominal scarring ($p < 0.01$), and obesity ($p < 0.02$). Two or more risk factors increased the fat necrosis rate to 24.7% compared with 8.3% in patients without risk factors ($p < 0.002$). Patients with multiple risk factors having bipedicled flaps did not have an increased risk for fat necrosis suggesting that the bipedicled procedure eliminated the impact of the risk factors by boosting flap blood supply. Our review of bilateral unipedicled TRAM flap reconstructions demonstrated no increased risk of fat necrosis or flap loss among bilateral patients [31]. Bilateral procedures showed a very slight increase in general complications such as atelectasis. Abdominal complications were not increased significantly. Kroll compared clinical and radiologic evidence of fat necrosis between 49 free and 67 pedicled TRAM flaps. The size of the lesions was not clear but all lesions were visible mammographically. Predictably, free TRAM flaps demonstrated an 8.2% incidence of detectable fat necrosis compared with 26.9% in pedicled TRAM flaps ($p < 0.01$). While fat necrosis was more common in obese patients and smokers, this did not attain statistical significance [58]. Elliott confirmed similar findings for their series of patients, but in all of these studies, measurement of the amount of fat necrosis has been very subjective [59].

Radiation impacts TRAM flaps causing both fibrosis and fat necrosis. In 1997, Williams reported the Emory experience with radiation administered either before or after TRAM flap reconstruction. Fibrosis within the reconstruction was found in 31.6% of radiated TRAM flaps but not in patients who received preoperative therapy. Fat necrosis was similar in both radiated groups at 17.6% and 10% in the nonradiated patients. Not surprisingly, obesity further compounded fat necrosis rates when coupled with radiation therapy [42]. Rogers found a similar trend when free deep inferior epigastric perforator flaps were exposed to postoperative radiation [60]. By contrast, Zimmerman reviewed 21 patients with free TRAM flap reconstruction and claimed little negative impact in the majority of patients [61]. The question of whether or not it is worthwhile performing a microsurgical turbocharged anastomosis to reduce fat necrosis has been addressed in a small series of patients by El-Mrakby [62]. Turbocharged pedicled flaps had almost twice the rate of fat necrosis of free flaps although the fact that these patients required turbocharging suggests sample bias. Their conclusion is that free flaps are superior to turbocharged pedicled flaps.

36.20 Abdominal Wall Strength and Contour After Pedicled TRAM Flaps

There has been considerable debate about the impact of pedicled versus free TRAM and DIEP flaps on abdominal wall function. It would seem intuitive that a free flap would have far less impact on abdominal wall function than pedicled flaps with bipedicled flaps demonstrating the worst outcome. In practice, this is not strictly true, particularly when activities of daily living are evaluated by the patients themselves. It appears that there is considerable recruitment of adjacent muscle power, and this tends to improve with time. Furthermore, it appears that even with free flap harvest, the residual rectus muscle tends to atrophy significantly and hernia rates are not that much less than with pedicled flaps. Hartrampf [11] reported a 1.5% hernia rate in 351 unipedicled TRAM flap reconstructions, while the Emory group reported a hernia rate of 8.8%, a figure strongly skewed by one surgeon's use of small inlay mesh repairs; this figure has since been reduced to approximately 3.9% [31, 32]. This is similar to the data presented by Petit from Milan reporting 251 TRAM flap reconstructions with a hernia rate averaging 7% now reduced to 2% [63, 64]. Paige's review of the Emory experience with 257 bilateral versus unilateral pedicled reconstructions over a 7-year period revealed no significant difference between the two groups in terms of abdominal morbidity. In a review of 268 patients who had undergone either free TRAM (FTRAM) or conventional pedicled TRAM (CTRAM) flap reconstructions at least 6 months before, Kroll found similar hernia rates whether unipedicled or bilateral flaps were harvested (3.8% vs. 2.6%, not statistically significant). Single pedicle free TRAM flap patients were more likely to perform sit-ups than conventional unipedicled flaps which in turn were more likely to be able to do sit-ups than bilateral free or bipedicled patients. The conclusion was that the abdominal hernia or bulge rate is independent of the type of TRAM flap used and the number of muscle pedicles harvested. By contrast, measured abdominal strength was affected by these factors as far out as 6 months postoperatively. Nahabedian evaluated 108 women with free TRAM flaps, 37 women with pedicled flaps, and 10 women

with DIEP flaps. Lower abdominal contour defects were far more common after bilateral free TRAM flaps than with DIEP flaps [65]. Blondeel found that free TRAM flaps impacted far more negatively on abdominal strength than did free DIEP flaps [66, 67], but even free DIEP flaps create abdominal weakness to some extent [68]. To further confound the issue, Suominen has performed several elegant studies to accurately measure abdominal strength and function up to 12 months postoperatively [23, 24, 69]. In a magnetic resonance imaging study of the residual rectus muscles left after free and pedicled flaps, the donor rectus muscle on the free flap side had atrophied by at least 25% when compared with the non-operated side, and fatty degeneration was significantly higher in the donor muscle. No hernias were detected in either group [24]. In another study by the same author, long-term follow-up of the pedicled and free TRAM flap groups was performed with a mean follow-up of 23 months. By this time, there were no significant differences in abdominal flexion/extension strengths between either group [23]. In a prospective study of 19 free versus 23 pedicled TRAM flap patients, Edsander-Nord assessed strength at 3, 6, and 12 months postoperatively. Apart from an initial transient decrease in strength (worse in pedicled than free flap patients), the strength differences resolved almost entirely by 12 months. What is interesting is that free TRAM flap patients experienced a greater incidence of lower abdominal bulging (82%) than their pedicled counterparts at 48% [70]. In a meta-analysis of previously published data, Reece and Kroll attempted to collate the evidence concerning abdominal wall morbidity after TRAM flap reconstruction. The data is interesting, but firm conclusions are difficult to arrive at given the widely disparate data collected [30]. In conclusion, it appears that the more muscle one harvests, the greater the initial impact on abdominal strength. As time progresses, pedicled and free TRAM flap patients develop very similar functional outcomes with little impact on the activities of daily living. Abdominal bulge and hernia rates appear to be independent of the type of flap harvested and may relate to the care with which repair has been undertaken as well as the quality of the fascia to be repaired. The exact mechanism for these observed differences has yet to be explained satisfactorily.

36.21 Total and Partial Flap Loss

While complete flap loss is extremely rare in pedicled TRAM flap reconstruction (2 of 350 unipedicled and 0 of 39 bipedicled TRAM flaps in Hartrampf's series) [11], partial flap loss is more common. Hartrampf reported an 8.5% incidence in his series, while Kroll reported a 15.4% incidence in slim patients increasing to 41.7% in obese patients [71]. Elliott reported a 10% incidence in a series of 128 cases of unipedicled TRAM flaps [59], and Trabulsy noted a 6% incidence of partial flap loss and 4% complete flap loss in their series of 99 patients [1]. By comparison, Chang reporting on over 700 free TRAM flap breast reconstructions found total flap loss in 5.1% with a 6.2% partial flap loss [72]. This pushes total flap necrosis-related complications to over 11% in a center of excellence. These figures should be borne in mind when occasional microsurgeons are tempted to embark on complex free flap procedures in higher-risk patients. Given the high patient satisfaction with pedicled TRAM flaps compared with DIEP flaps [73], this may also explain why many surgeons who are comfortable with microsurgery are reluctant to convert to performing free TRAM or DIEP flaps routinely in their practices, given the time and cost restraints of these complex procedures.

36.22 The Impact of Obesity on TRAM Flap Viability

The most comprehensive study to date detailing the impact of obesity on human flap viability is that presented by Chang et al. [72]. In this study alluded to above, free TRAM flap results were evaluated based on the patient's body mass. Normal-weight patients ($n = 442$) had no total flap losses and a 1.6% partial flap necrosis rate. Overweight patients ($n = 212$) experienced 1.9% total flap loss with a 1.4% partial flap necrosis rate. By contrast, 64 obese patients had a 3.2% total flap necrosis rate and 3.2% partial flap necrosis. Fat necrosis rates were 6.1% in normal patients, 9% in the overweight group, and 7.8% in the obese category. Abdominal bulges were three times more common in overweight patients compared with normal, and seromas were ten times more common in obese patients. In the Emory University study of 556 patients, obesity correlated with both fat necrosis and general complications at the $p < 0.02$ level [32].

36.23 Smoking and TRAM Flap Viability

Watterson's study demonstrated a significant correlation between smoking and general complications ($p < 0.002$), but interestingly smoking did not correlate strongly with fat necrosis [32]. Hartrampf accorded heavy smoking a moderate risk in his scoring system for TRAM flap patient selection criteria [11]. Chang found a significant risk for both the reconstruction and the donor site in smokers compared with non-smokers, with those having more than a ten-pack-year history faring worse than those with shorter histories. Former smokers and non-smokers had similar complication rates [72]. In another study, Padubidri found overall complications to be greater in smokers at 39.4% versus 25% in ex-smokers and non-smokers [74].

36.24 The Timing of Reconstruction in Relation to Radiation Therapy

In the past, radiation therapy had been reserved for those patients with more advanced breast cancers and more than three positive axillary lymph nodes. The publication of two papers, one from Denmark and the other from Canada, initiated a major swing toward treating early breast cancer patients with adjunctive radiation in an effort to improve survival [75, 76]. The result has been that more and more patients with TRAM flap reconstructions are now facing postoperative radiotherapy and then facing the consequences of radiation's impact on the flap. Add to this the dramatic impact of skin-sparing mastectomy on breast reconstruction and one can see what a dilemma the reconstructive surgeon now faces. Should the patient who faces radiotherapy in her future proceed with mastectomy first and then have delayed reconstruction, or should we go ahead with a skin-sparing mastectomy with all of its benefits, reconstruct the breast with a TRAM flap, and then proceed to radiation accepting its negative consequences? This dilemma is the subject of constant debate at national and international meetings. All of us who frequently perform TRAM flaps are aware of radiation's impact on these flaps, whether pedicled or free. TRAM flaps tolerate radiation better than expander-implant reconstructions and with fewer complications [77]. Williams reviewed the Emory experience with radiation and found it to increase fibrosis as well as fat necrosis depending on the timing of treatment in relation to surgery [78]. Flap loss was not increased per se, a finding corroborated by Kroll's review of 428 flaps (of 1384 free flaps total) transferred to previously radiated beds [79]. It was Kroll's belief that radiation significantly impacts the feel and shape of TRAM flaps when administered after reconstruction as evidenced by William's data. His conclusion was that patients in whom radiotherapy is likely post-mastectomy should complete their radiation and then proceed to TRAM flap reconstruction, forgoing the benefits of skin-sparing mastectomy and immediate reconstruction. In this manner, the final reconstruction may be spared the deleterious effects of radiation injury in the long term even though there is a greater likelihood that such patients may need free or bipedicled TRAM flap procedures. There is certainly merit in this argument given the possible prospect of fibrosis, distortion, and fat necrosis that may supervene in a radiated TRAM flap.

36.25 Pregnancy Following Pedicled TRAM Flaps

Despite the loss of muscle function after pedicled TRAM flap harvest, it is still possible for patients to conceive and carry a pregnancy to term as well as achieve normal vaginal delivery [80]. Johnson described the successful vaginal delivery of monozygotic twins after bilateral pedicled TRAM flap reconstruction [81] indicating that patients can be reassured that their abdomens will in all likelihood perform satisfactorily even under the considerable stress of twin pregnancy. Parodi cautions against patients becoming pregnant within 12 months after TRAM flap surgery, reporting a single case of a woman becoming pregnant at 4 months postoperatively and developing a hernia. She delivered vaginally at term [82].

36.26 Patient Satisfaction Outcomes

A patient's emotional outcome after breast reconstruction is unpredictable and highly individual [83]. Several factors influence the aesthetic outcome [84]. In a study of 125 women diagnosed with breast cancer, Keith found that 49.6% of his respondents desired breast reconstruction if available. Young women and depressed women favored reconstruction more than older patients. In Keith's study, marital status, tumor size, extrovertism, neuroticism, and tough-mindedness were not independently predictive of the desire for reconstruction [85]. Of patients requesting reconstruction, 63% were concerned that reconstruction might mask recurrence, but 94% felt that it would greatly benefit their self-esteem. Age does not appear to be a significant risk factor for pedicled TRAM flap usage as evidenced by a study of 84 patients aged 65 years or older in whom successful reconstruction was achieved [86]. In another study evaluating patient acceptance of the procedure, Nissen found that while women were highly satisfied with their reconstruction, their greatest anxiety remained the fear of recurrence as well as a desire to be as informed as possible about complications and recovery [87]. This was reinforced in a study by Tykka who found most women were highly satisfied with their TRAM flap reconstructions, all of which in this study had been performed to replace inconvenient bra prostheses [88]. The patients were particularly pleased with the autologous nature of the reconstructions but had been surprised by the extent of the surgery and length of the recovery process. This highlights the importance of warning patients that recovery will take a minimum of 3 months before patients start to feel as if life is returning to normal once more. It appears that patients are more accepting of the quality of their reconstruction than are their surgeons as evidenced by a study of 20 patients whose level of satisfaction was much higher than that of their surgeons [89]. In another study of 60 inner city women undergoing breast reconstruction, demographic studies failed to show any differences in education, economic status, or insurance status in women undergoing reconstruction. In this study, reconstructed women had a higher satisfaction with their sex lives and body image than did non-reconstructed women [90]. While these trends are culled from relatively

small patient populations, it is apparent that breast reconstruction can be an immensely satisfying procedure for both patient and surgeon and can have a positive impact on a patient's daily life and convenience.

Conclusion

Pedicled TRAM flap breast reconstruction remains a common choice for autologous reconstruction and is readily learned by any competent surgeon. It provides excellent contour and softness in most patients and abdominal complications are few. Given the potential for free flap failure and the added cost involved in additional operating time for microsurgical procedures [91], pedicled TRAM flaps remain the most cost-effective method of autologous breast reconstruction in most surgeons' hands [91]. Although TRAM flap reconstruction is a major operative procedure, it provides both patient and surgeon with a unique tool to achieve a natural, soft, warm, well-integrated reconstruction after mastectomy.

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Breast Reconstruction with Free Flaps

37

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37.1 Introduction

In 1973, Taylor and Daniel used the term "free flap" to describe the distant transfer of an island flap by microvascular anastomosis [1, 2]. They also carried out a detailed anatomical description of many of the more common free flap donor sites still in use to date [3].

The modern era of autologous breast reconstruction began with the free abdominoplasty flap introduced by Holmström in 1979 [4] and with the transverse rectus abdominis myocutaneous (TRAM) flap by Hartrampf in the early 1980s [5]. Both used the common pedicle flap concept to transfer autologous tissue from the abdomen to the chest wall for breast reconstruction using the inferior epigastric artery for the former and the superior epigastric artery for the latter with the rectus abdominis muscle as a carrier [4, 5].

Because of the complications correlated with the use of transverse rectus abdominis muscle such as abdominal wall weakness, abdominal bulging, frank herniation, and abdominal and lumbar back pain, the concept of donor-site muscle-sparing techniques was then embarked upon. On this basis Koshima used the skin territory overlying the rectus abdominis muscle to reconstruct head and neck defects [6]. The flaps were based on a single paraumbilical perforator vessel from the deep inferior epigastric artery. The perforator vessels were dissected meticulously leaving an intact rectus abdominis muscle and followed toward the deep inferior epigastric vessels to achieve adequate pedicle length. The resulting flap was thin, composed only by the skin and the vascular pedicle. The goal of muscle preservation became more apparent, and the next significant step was related to the work on perforator flap of the authors' group at Louisiana State University Medical Center. They injected fresh abdominoplasty specimens assessing that the skin and fat could be transferred without the use of the rectus abdominis muscle. This led to the realization of the first deep inferior epigastric perforator (DIEP) flap for breast reconstruction by Allen in 1992 [7]. By the use of this original procedure, 15 breasts were successfully reconstructed offering the same benefits as the TRAM flap but reducing the abdominal wall morbidity. The beginning of free tissue transfer allowed an infinite range of possibilities to appropriately match donor and recipient sites.

It is known that the type and timing of reconstruction is a multifactorial decision. It is based on the size and shape of the native's breast, location and type of cancer, patient's characteristic, patient's expectations whether adjuvant radiation and/or chemotherapy are needed, and the surgeon's preferences and skills. The commonly used autologous tissue flaps for breast reconstruction are free TRAM (muscle sparing) flap, DIEP flap, superior gluteal artery perforator (SGAP) flap, inferior gluteal artery perforator (IGAP) flap, transverse upper gracilis (TUG) flap, and superficial inferior epigastric artery (SIEA) flap. The alternative flaps, e.g., Ruben's flap (DCIA), anterolateral thigh (ALT) flap, and transverse fascia lata (TFL) flap, are occasionally used for breast reconstruction.

37.2 Free Flaps from Abdomen

37.2.1 Free TRAM Flap

The skin paddle of the TRAM flap is drawn between the umbilicus and pubic region and from the front of the iliac bone. Both deep superior and inferior epigastric vessels supply the muscle, while the skin and fat tissue are vascularized by perforators through the rectus abdominis muscle. The flap can be harvested either in a free microvascular or in a pedicled form. In the pedicled form, the caudal portion of the muscle is detached. Still left connected to the rest of the muscle, the flap is moved under the skin up to the mastectomy

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site to reconstruct the breast. The modern free TRAM as "free abdominoplasty flap" for breast reconstruction was described first by Holmström in 1979 [4]. As a free microvascular flap, the TRAM flap is harvested with a small cuff of the rectus abdominis muscle and brought to the mastectomy site reestablishing the blood supply by microsurgical anastomoses between the deep inferior epigastric pedicle and the scapular circumflex vessels. Based on the amount of rectus abdominis muscle spared [8], the free TRAM is classified into four types: in MS0, full width (partial length) of the rectus abdominis muscle is harvested; in MS-1, lateral segment is preserved and as a result the innervated lateral rectus muscle is left intact. The middle/medial rectus muscle just enough to support the perforators with a narrow strip of anterior sheath is harvested with the flap. In MS-2, lateral and medial segment is preserved, while MS-3 is equivalent to DIEP flap because the entire muscle is left attached. The abdominal complications are significantly reduced with this technique compared to the pedicled TRAM flap as both the lateral innervated muscle and rectus sheath are preserved. Compared to the pedicled TRAM flap, reconstruction of breasts with the free TRAM flap offers a lower complication rate at the mastectomy site and a low donor-site morbidity rate as reported by Kroll [9] even if the ability to do sit-up from supine position was best retained in free TRAM (63%), followed by pedicled TRAM (57%) and free bilateral TRAM (46.2%), and worse in pedicled bilateral TRAM.

In literature, a lot of studies reported the impact of free flap breast reconstruction on the abdominal wall in order to minimize donor-site morbidity [10–14].

The planning of the free TRAM flap is similar to the DIEP flap. All details are reported in the next subheading.

37.2.2 DIEP Flap

The flap harvesting includes the identification of at least one reliable perforator. The average pedicle length is 8–15 cm and diameter is 2–3.6 mm. The first step is to assess the amount of skin and subcutaneous adipose tissue that should be transferred from the abdomen to the mastectomy site to achieve symmetry in ptosis and volume with the opposite breast. A simple pinch test can help the surgeon to estimate the amount of excess tissue available for reconstruction. The use of a complementary device in surgeon evaluation such as the BREAST-V [15] can be useful to predict preoperatively the volume of the breast that has to be reconstructed. An app entitled BREAST-V for both iOS and Android devices is currently available for free download in the Apple Store and Google Play Store.

Preoperative markings are done with the patient in upright position. The general surgeon indicates the oncological planning and the breast skin area that has to be removed with the mammary gland. Plastic surgeon then can plan the DIEP flap harvest. Standard abdominoplasty markings are made in the sitting or standing position, while the flap markings included vascular zones I–III for unilateral and zones I–II for bilateral reconstructions according to Holm et al. [16]. The side of the abdomen contralateral to the side to be reconstructed is usually preferred as it allows simultaneous operating by two teams. The inferior incision is marked in the natural suprapubic crease and extended to the level of the anterior superior iliac spine (ASIS) on both sides, while the superior incision is made joining the two ASIS passing over the umbilicus. The umbilicus is first dissected and the superficial inferior epigastric vein is identified and preserved if significant in size. Depending on the type of mastectomy that should be performed, the flap can be de-epithelialized during this step as planned. Then the flap dissection starts in a lateral-to-medial direction above the deep fascia until the first reliable perforator of the lateral/medial row is visualized. The number of selected perforators depends on the perforator's position, vein diameter, and flap volume to be transferred. The superficial inferior epigastric vein can be preserved and included in the flap as an extra-venous outflow.

Rectus sheath is opened around the perforator to facilitate further dissection following its course until its origin. Only the part of the rectus abdominis muscle that is found among the vessel is sacrificed in order to identify and dissect the course of the deep inferior epigastric pedicle running under the muscle until its origin from the external iliac vessels. Care is taken to preserve any intercostal nerves innervating the medial aspect of the muscle that might cross the pedicle. The superior end of the pedicle is divided above the perforator, and the zone IV of the flap is discarded. Once the recipient vessels are ready, the inferior end of the pedicle is ligated. The flap is then weighed and transferred, rotated 180°, and fixed temporarily to the chest wall. Great care is taken for the flap pedicle position without any twists or kinks; both arterial and venous end-to-end anastomoses are performed, while a second team carries out donor-site closure. The anterior rectus sheath is repaired with interrupted 1-0 vicryl sutures, and the umbilicus is relocated at a level above the ASIS. The Scarpa's fascia is approximated with interrupted 2-0 vicryl sutures, while the abdominal skin is sutured in two layers. The abdominal wall is supported by an elastic band, and the patient is positioned on the bed in half-sitting position in the immediate postoperative period. Next, the flap insetting is completed while the contralateral procedure is performed if already planned (Figs. 37.1, 37.2, 37.3, 37.4, and 37.5).

Although good and long-term aesthetic outcomes can be obtained in unilateral DIEP flap reconstruction, contralateral procedures are often required to achieve breast symmetry. Stevenson and Goldstein compared TRAM flap and immediate contralateral breast reduction/mastopexy with TRAM flap alone observing a satisfactory aesthetic result [17]. Haykel and Gay reported a single-stage autologous reconstruction by the use of both pedicled flaps and free

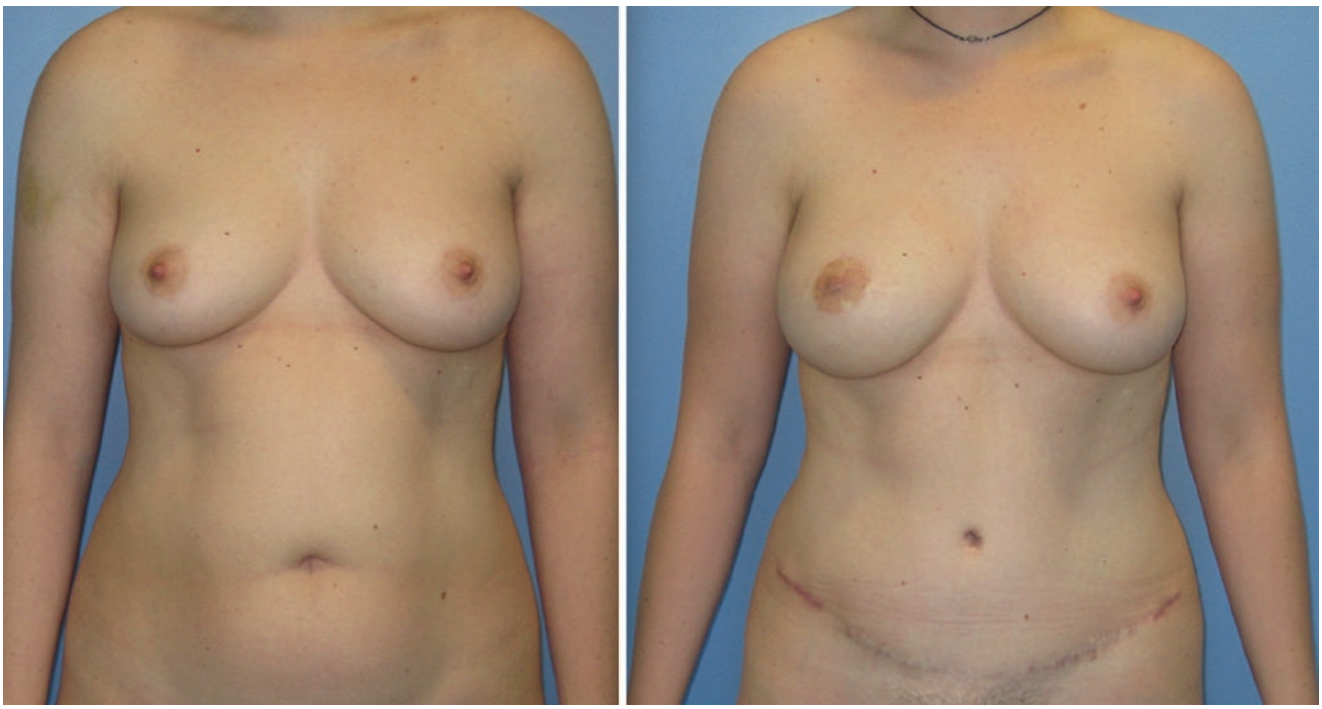

Fig. 37.1 A woman underwent immediate one-stage DIEP flap reconstruction following right nipple-sparing mastectomy. Preoperative (*left*) and postoperative (*right*) frontal view

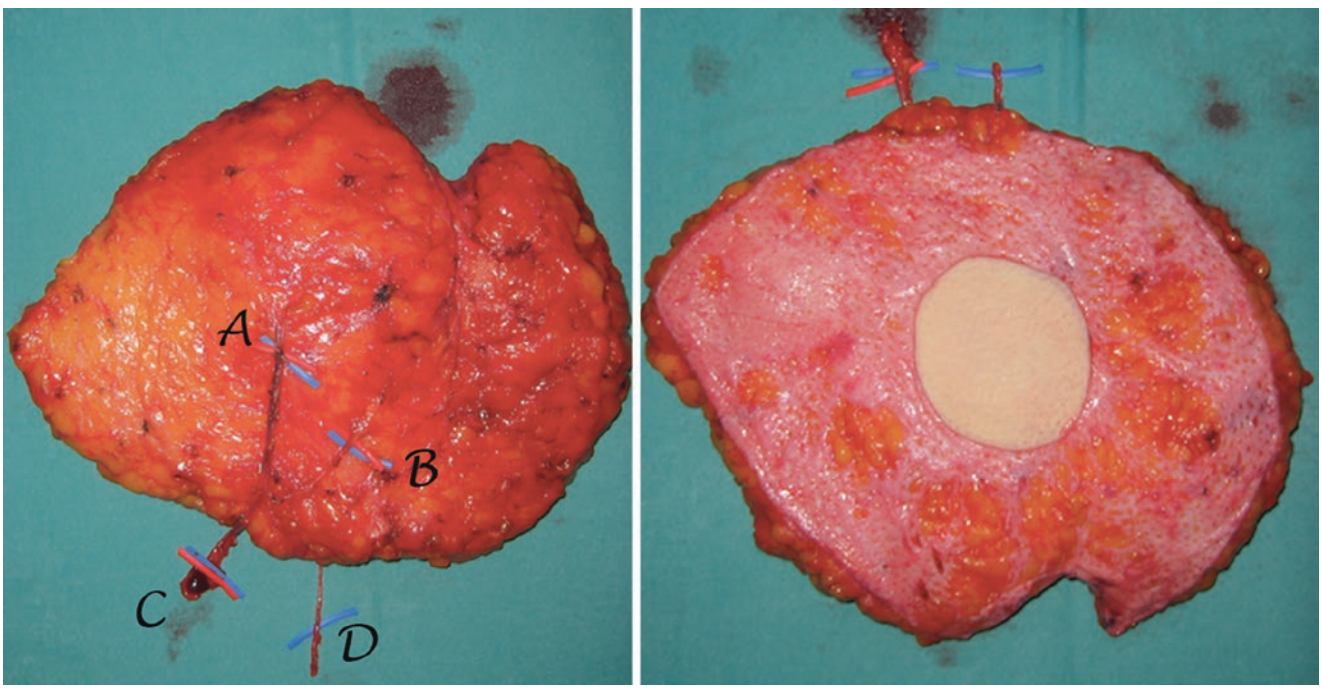

Fig. 37.2 Intraoperative DIEP flap. Posterior view (*left*) showing two perforators, superficial epigastric vein and deep inferior epigastric pedicle. Anterior view (*right*) showing sentinel skin island

flaps. They observed 11.3% of postoperative aesthetic complications in 141 patients by means of volume excess (5.7%), malposition (2.1%), volume loss due to weight loss (1.4%), shape asymmetry (1.4%), and volume deficiency (0.7%) [18].

Huang et al. observed greater patient satisfaction, minimal increase in operative time, and no increase in complication rates comparing clinical and aesthetic outcomes in immediate and staged contralateral surgery in DIEP and superficial inferior epigastric artery (SIEA) flap reconstruc-

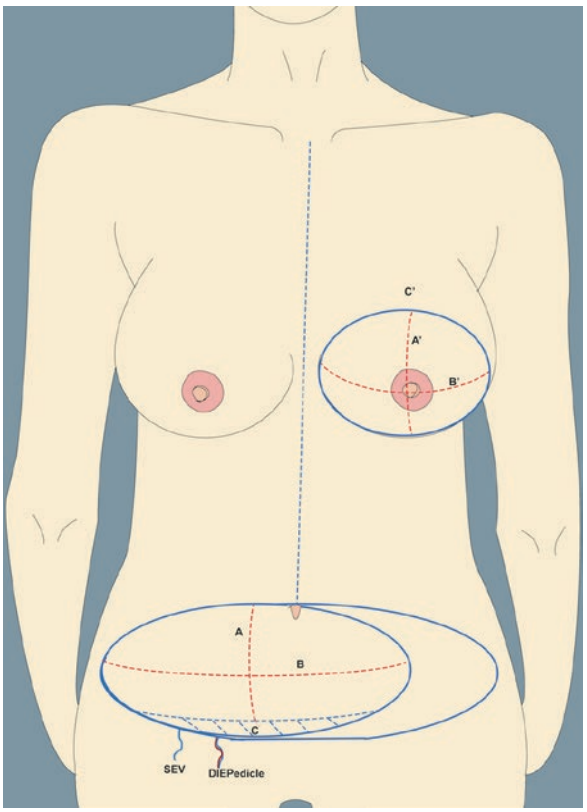

Fig. 37.3 DIEP flap breast reconstruction planning. Modified radical mastectomy is drawn on the affected breast (A = vertical axis, B = horizontal axis). DIEP flap markings with a skin paddle (A′ = vertical axis, B′ = horizontal axis) to match the mastectomy pattern (A = A′, B = B′) and the de-epithelialized area of the flap (C, C′). *SEV* superficial epigastric vein, *DIEPedicle* deep inferior epigastric pedicle

tion [19]. One-stage DIEP flap reconstruction by means of the symmetrization algorithm was also described resulting in comparable aesthetic outcomes and patient satisfaction to a staged procedure [20].

37.2.2.1 Recipient Vessel Selection

The choice of recipient vessels is one of the key points in microvascular breast reconstruction, and it is largely up to the reconstructive surgeon and usually based on comfort level and experience, flow characteristics, chest topography, and patient comorbidities. In the recent literature, there is no unanimously agreed upon recipient sites for anastomoses. Both internal mammary vessels (IMV) and axillary vessels such as thoracodorsal (TDV) and circumflex scapular vessels (CSV) are usually easy to expose and of suitable caliber, allow an end-to-end anastomosis, and have demonstrated advantages and disadvantages in this setting. Some surgeons advocate the use of IMV as recipient vessels of choice [21–24], while others observed unpredictable quality and inconsistency of the internal mammary vein diameter at the level of the fourth rib, difficult anastomosis due to respiratory movements, risk of pneumothorax, and contour deformity due to rib cartilage excision [24]. Time for vessels' dissection and exposure in immediate reconstruction represents an issue in favor of the axillary vessels. The general surgeon following the lymphadenectomy or sentinel lymph node biopsy performs the preparation of these vessels, while the access to the IMV usually needs an extra "step" [25].

Previous reports have demonstrated that the flow rate of the flap is correlated to its size and therefore its flow rate is

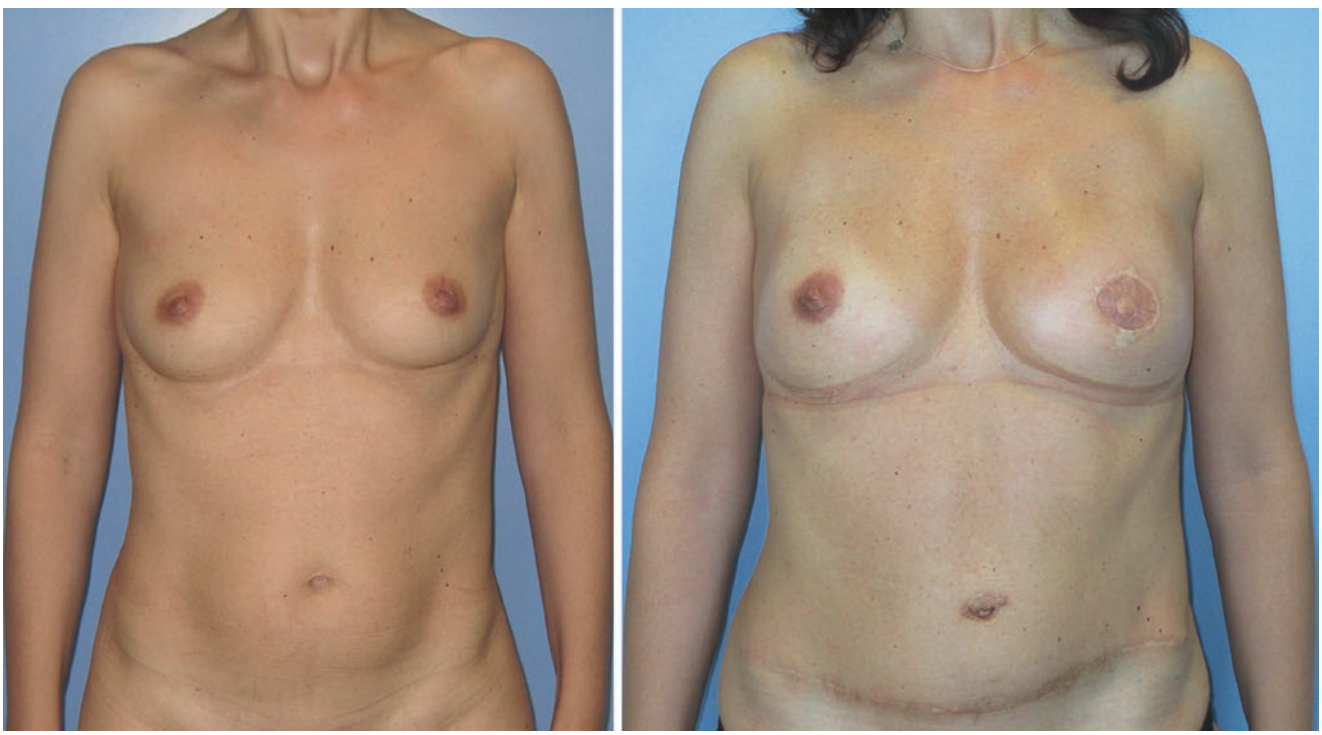

Fig. 37.4 A woman underwent immediate bilateral one-stage DIEP flap reconstruction following right nipple-sparing mastectomy and left skin-sparing mastectomy. Preoperative (*left*) and postoperative (*right*) frontal view

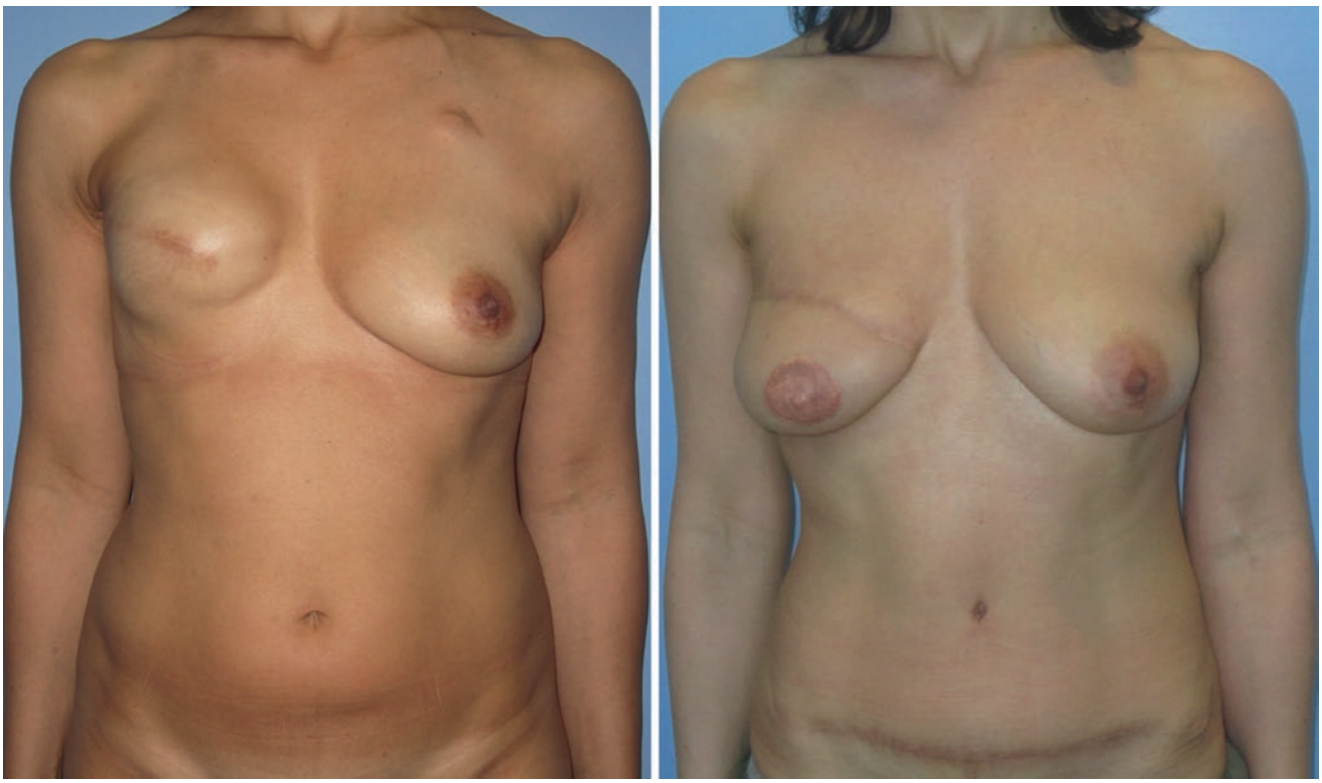

Fig. 37.5 A woman underwent secondary DIEP flap breast reconstruction after right modified radical mastectomy and failed reconstruction with breast expander. Preoperative (*left*) and postoperative (*right*) frontal view

correlated to its venous drainage [26, 27]. Adding veins in parallel and choosing veins with larger diameter can increase drainage from the flap and reduce risk of venous congestion. Both axillary and IMV have adequate negative vein pressure and drainage, but using IMV the main drawback is the difficulty to have two veins of adequate size and length compared to TDV and CSV [27, 28].

The perforators arising from the internal mammary vessels have also been used as recipient vessels avoiding many of the complications related to the use of IMV, but unfortunately their presence depends on anatomical variability and preservation by the general surgeon [29]. The absence of a proper and careful preoperative planning may cause an increased operative time and/or surgical errors.

We prefer the use of the CSV as a recipient pedicle [28]. Majority of our cases involve immediate breast reconstruction where the axillary vessels are already exposed during lymph node dissection. From our experience, vascular anastomosis, re-exploration, and revision are technically easier in the axilla. Complications such hematoma can be quickly identified and managed easily in the axilla. Moreover the use of CSV saves TDV pedicle to assure the viability of a myocutaneous latissimus dorsi flap, useful for salvage procedure in case of previous reconstruction failure. Furthermore, despite the possibility of accidental TDV injury by the general surgeon during lymph node dissection or radiotherapy damage in delayed reconstructions, the anatomical location of the CSV make it unlike for CSV [23, 30] as they are located in the omotricipital space and not embedded in scar tissue neither their lumen is never reduced nor weakened.

37.2.3 SIEA Flap

The procedure is similar to the DIEP flap, but the SIEA flap is harvested on the superficial inferior epigastric vessels just below the skin surface avoiding injury to the anterior rectus abdominis fascia or the muscle. Grotting first described it for breast reconstruction in 1991 [31]. The SIEA artery is a direct cutaneous vessel that arises from the medial side of the common femoral artery as a common trunk with superficial circumflex iliac artery or, independently, approximately 2 cm below the inguinal ligament. It travels up and pierces the cribriform fascia supplying the subcutaneous tissue and skin of the lower abdomen. The artery is accompanied by venae comitantes, and an additional prominent vein runs 3–4 cm medially to the artery. As previously described, this vessel is very useful as a second vein anastomosis in DIEP flap reconstruction.

By the use of the SIEA flap, there is no risk of abdominal hernia or bulge. Indeed, there is the significant saving of operating time because the vessels run above the rectus sheath avoiding any tedious intramuscular vessel dissection. Nevertheless, its major drawbacks are the inconsistent vascular pedicle anatomy, the shorter (6–15 cm) and smaller

diameter (1.4 mm) vascular pedicle, and limited flap territory. The skin area supplied by the artery is 140 ± 100 cm^2 running in a curvilinear way, 5 cm above the iliac crest. The flap spreads from the anterior superior iliac spine to the lateral border of the rectus abdominis muscle and below the umbilicus to the pubic hairline [32, 33]. A well-vascularized flap includes the zones I and II (ipsilateral hemiabdomen), while zones III and IV (contralateral hemiabdomen) are not considered reliable. Blondeel observed the inverse relationship between superficial and deep inferior epigastric veins [34]. The superficial inferior epigastric vessels may be dissected and preserved if more than 1.5 mm in diameter as previously suggested, irrespective of DIEP, SIEA, or MS-1 TRAM [35, 36].

37.2.4 Abdominal Free Flaps and Prior Abdominal Surgery

Despite the relative contraindications due to the supposed extensive vascular interruption [5, 37, 38], free TRAM, DIEP, and SIEA flaps can be harvested safely by tailoring the flaps previous abdominal surgery such as appendicectomy, hysterectomy, low cesarean section, subcostal cholecystectomy, midline laparotomy, laparoscopy, and other laparoscopic scars. Hamdi et al. proposed their clinical approach to maximize the perfusion to the upper abdominal flap by either widening/lengthening or "augmenting" the supply of the upper flap (with perforator preservation). A bipedicled free flap is also suggested in order to obtain an adequate amount of tissue when the anatomical condition does not allow harvesting ample tissue using standard flap markings [39]. Depending on the location and type of the scars, available vascular pedicle, perforator locations, and required flap tissue for breast reconstruction, Laporta et al. also suggested three clinical approaches by the use of standard abdominoplasty markings to ensure well-vascularized flap and to minimize abdominal donor-site complication [40]. If the Pfannenstiel or laparoscopic (hysterectomy or other gynecological operations) scars damage or interrupt the deep inferior epigastric vessels, the length of the pedicle is too short to reach CSV; they suggest the use of the serratus anterior pedicle (SAP) as recipient vessels. In patients with midline laparotomy scars, a simple but effective strategy is to vertically split the infraumbilical tissue into two hemi-DIEPs. To overcome the relative lack of volume, they propose a delayed fat graft session within 6 months after primary surgery. In all patients with a chevron or subcostal scars, the skin area caudal to the previous incisions is left attached to the periumbilical perforators.

In doubtful cases, a CT angiogram may be useful and can be arranged preoperatively to assess the status of perforators and the main vessels.

37.3 Free Flaps from Other Sites

In cases where abdominal tissue is not available for reconstruction, focus shifts on the gluteal or upper thigh region as donor sites. The SGAP, IGAP, TUG, and profunda artery perforator (PAP) flaps are usually considered flaps of second choice when abdominal tissue is not available. In a review of 31 cases of GAP by Granzow et al., 20 (65%) were performed due to inadequate abdominal tissue, 6 (19%) due to patients' donor-site choice, and 2 (8%) due to failed DIEP flaps [41].

37.3.1 SGAP Flap

The superior gluteal myocutaneous free flap for breast reconstruction was first described by Fujino in 1975 [42] and popularized by Shaw [43]. The main limitation of this flap is the short vascular pedicle, which frequently requires vein grafting, increasing the difficulty, complications, and the time required for this procedure. The SGAP flap for breast reconstruction was described by Allen et al. in 1993 [44]. Advantages of the gluteal artery perforator flaps versus the previous myocutaneous gluteal flaps include the preservation of the gluteus maximus muscle and additional length of the pedicle without the use of vein grafts for anastomosis.

The superior gluteal artery (SGA) is a continuation of the posterior division of the internal iliac vessel. It is a relatively short artery (6–8 cm) and emanates from the pelvis above the upper border of the piriformis muscle, where it immediately divides into both superficial and deep branches. The deep branch travels between the gluteus medius muscle and the iliac bone, while the superficial branch supplies the upper portion of the gluteus muscle and overlying fat and skin.

With the hip slightly flexed and rotated inward, a line is drawn from the posterior superior iliac spine to the posterior superior angle of the greater trochanter. The point of emergence of the superior gluteal corresponds to the junction of the upper and middle thirds of this line. An average of four perforators is usually found from the superior branch of the SGA to supply the skin.

The skin paddle can be designed as an oblique ellipse or horizontally producing a more favorable scar keeping the marked perforator in the center. The average flap height and length are 8 cm (range 5–12 cm) and 22 cm (range up to 30 cm). It should be noted that perforators located laterally would produce longer pedicles. The whole flap can be raised on single perforator and the donor site is closed primarily.

In unilateral cases, the patient is positioned in the lateral decubitus in order to perform mastectomy, recipient vessel

dissection, and the flap harvest simultaneously. If a bilateral procedure is scheduled in the primary setting, the patient is placed first supine during the mastectomy and exposure of the recipient vessels. Then, prone positioning, flap harvesting, and closure of both buttocks follow this dissection. The patient is returned in supine position and redraped in order to complete the anastomosis in this setting. The flap is elevated from the muscle in the subfascial plane, and the perforator's dissection starts from lateral to medial. It is preferred to use a single large perforator, if present, but two perforators in the same plane and direction of the gluteus maximus muscle fibers can be taken together as well. The muscle is then spread in the direction of the muscle fibers, and the perforators are followed through the muscle. The dissection continues until both the artery and the vein are of sufficient size to be anastomosed to the recipient vessels in the chest wall.

37.3.2 IGAP Flap

Higgins et al. subsequently introduced the IGAP flap for ischial pressure sore reconstruction in 2002 [45], while Guerra et al. introduced it for breast reconstruction in 2004 [46]. This flap uses the excess lower buttock tissue and preserves the muscle leaving the scar in the natural depression of the inferior gluteal crease. Its use is not widespread, and in literature, there are few articles encouraging this procedure for breast reconstruction [47, 48].

Inferior gluteal artery is the terminal branch of the anterior division of internal iliac artery and emerges through greater sciatic foramen below the piriformis muscle, with sciatic nerve and posterior cutaneous nerve of the thigh. The course of the vessel is more oblique providing a longer pedicle than the SGAP flap.

The gluteal crease is marked with the patient in the standing position. The flap is drawn as a horizontal ellipse with the major axis centered above the gluteal crease and the inferior incision located 2 cm inferior to it. The orientation of the skin paddle is usually parallel to the gluteal fold.

A second line is drawn from the posterior superior iliac spine to the outer part of the ischial tuberosity; the junction of the lower and middle third marks the point of emergence of the inferior gluteal arteries.

The dimensions of the flap usually are typically 8 cm (maximum 12 cm) in length and 18 cm (maximum 30 cm) in width. Flap dissection and patient positioning on operating table is similar to SGAP flap planning. Care must be taken to avoid injury to the posterior femoral cutaneous nerve of the thigh that travels with the inferior gluteal vessels. The sciatic nerve is never visualized because of the subfascial dissection. In small-breasted patients and large buttocks, the IGAP flap can give an excellent result, particularly with secondary liposuction of the lateral thighs. Nevertheless, if a unilateral small IGAP flap is harvested in a thin patient, buttock projection and the inferior gluteal fold may be distorted with unattractive outcomes. Painful donor-site scar and discomfort particularly in the sitting position are more common with the IGAP harvest than the SGAP.

37.3.3 TUG Flap

In 1992, Yousif et al. described the use of the transverse gracilis musculocutaneous flap for breast reconstruction with a transverse orientation of the cutaneous paddle in the proximal third of the muscle [49]. Arnez and Wechselberger popularized simultaneously the flap presenting their case series in 2004 [50, 51]. Compared to the common free flaps from the abdomen (TRAM, DIEP) and the buttocks (SGAP, IGAP), the size of the free TUG flap (25 cm × 10 cm) is smaller and the fat pad is usually thinner [51]. Because of the aforementioned, the flap provides inadequate tissue bulk to reconstruct large and ptotic breasts. Moreover, it is also considered less appropriate for secondary reconstructions where additional skin may be required for the excision of tissues in between the postmastectomy scar and the new inframammary fold.

With the patient in the supine position and the knee and hip flexed, the lower extremity is abducted. The flap is harvested from the ipsilateral side. This enables the reconstructive and general surgery teams to work simultaneously. The skin paddle is oriented transversely on the upper part of gracilis muscle just below the groin crease. The average pedicle length is 5–6 cm, with the vessel diameter around 1.6 mm. A line is drawn on the medial thigh from the pubic symphysis to the medial tibial condyle.

On this line, 8–12 cm distal to the symphysis, perforators from the main (proximal) vascular pedicle are identified by the use of a Doppler probe and marked. Around this marking, the width (vertical diameter) of the flap usually depends on the excess of skin/fat that allows primary skin closure avoiding the risk of wound dehiscence.

The flap is then outlined as a crescent shape with one tip in the lateral anterior groin and the other tip in the middle of the proximal posterior thigh. The superior incision of the crescent is parallel to the groin-gluteal crease (4 cm distal to it), while the inferior incision is drawn 10–12 cm distally.

The incisions are made along the preoperative markings, and the flap is dissected from an anterior to posterior direction in a subfascial plane until the intermuscular septum between the adductor longus and the gracilis muscle is visualized.

The greater saphenous vein can be included in the flap harvest allowing an extra-venous drainage. The fascia is incised anteriorly at the posterior border of the adductor lon-

gus muscle, which is then retracted. The dominant vascular pedicle is exposed traveling under the adductor longus and medially the undersurface of the gracilis muscle. The vascular pedicle dissection proceeds until its origin from the profunda femoris artery. The fascia is then incised at the posterior border of the gracilis muscle, and at the knee-joint level, the tendon of the gracilis muscle is divided and mobilized proximally. After checking vascular flap perfusion, the pedicle is divided and then ligated and the TUG flap is transferred to the chest wall. Both the artery and vein from the dominant gracilis pedicle are usually anastomosed end to end to the IMV or TDV. The donor-site closure is similar to the tight lift.

37.3.4 PAP Flap

An interesting recent advance of the TUG flap has been the description by Allen et al. of the profunda artery perforator (PAP) flap [52].

The ideal patient has a breast of small-medium size and excess tissue in the posterior thigh with contraindication for DIEP flap reconstruction. Preoperative imaging such as magnetic resonance or computed tomographic angiography of the pelvis and thigh with contrast can be useful to identify suitable posterior thigh profunda artery perforators. The posterior thigh tissue is bordered horizontally by the iliotibial tract and adductor muscles and vertically by the gluteal fold and popliteal fossa. The profunda femoris artery enters the posterior compartment of the thigh proximally to the knee giving three main perforators. The first perforator supplies the adductor magnus and gracilis, and the second and third perforators supply the semimembranosus, biceps femoris, and vastus lateralis. By the use of the imaging and a handheld Doppler, the perforators are identified and marked on the skin. Between medial and lateral perforators, the medial perforators are preferred because of the easier harvesting in the supine position and the perforator size. The skin paddle is drawn as an ellipse avoiding visible scar in the lateral or medial portion of the thigh outside of the gluteal fold. The superior marking is 1 cm inferior to the gluteal crease, while the inferior marking is approximately 7 cm below it.

Flap harvest can be performed in the prone or supine position. The supine frog-leg position offers the advantage of rapid dissection from a medial approach. Moreover, it is not necessary to reposition the patient to decrease the operative time. The prone position uses a lateral approach and gives the possibility to convert the PAP in TUG flap if no adequate perforators are identified. The elliptical incision is made and dissection proceeds in a suprafascial plane that helps with perforator identification. Once the key perforator is found, standard perforator dissection proceeds to harvest the desired pedicle length and vessels diameter. The donor-site closure is carried out in a multilayer fashion over a drain. After recipient-site preparation, the flap is transferred to the chest wall and the anastomoses are performed. The advantages on the use of PAP flap compared to TUG flap include the pedicle length as long as 13 cm (average, 9.9 cm) that provides versatility at the recipient site (IMV or TDV or CSV), the elliptical design that provides an ideal shape for coning to create a natural breast without the gracilis muscle harvest, and lower risk of lymphedema and seroma compared to the TUG because of the dissection near the lymphatic system that is not damaged. Moreover, the posterior femoral cutaneous nerve can be used to transfer a sensitized flap with branches from this nerve.

37.4 Complications

Vascular complications in free tissue transfer cannot be completely prevented; however a careful preoperative evaluation, prophylactic strategies, meticulous surgical technique, and meticulous postoperative monitoring may be significantly reduced the incidence [53]. Rapid exploration with revision is possible if an adequate flap monitoring is performed in the early postoperative period. Venous complications are more common than arterial likely due to the venous low-flow system being more prone to stasis or the easy vein kinking or compressing [53–56].

Kroll et al. reported that all microvascular complications occurred within the first 48 h and recommended that 3–4 days was the optimal length of time required for intensive postoperative microvascular monitoring [57].

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Contralateral Breast Management

38

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38.1 Introduction

Breast reconstructive surgery techniques have increasingly evolved in the last decades both by a surgical and postoperative management point of view.

More and more attention has been paid on the quality of life of patients undergoing this kind of surgery. Nowadays a reconstructive result can be considered worth not only when an acceptable morphology of the reconstructed breast alone is achieved but symmetry with the contralateral breast in terms of volume and shape obtained.

As a consequence, oncoplastic surgeons are paying more and more attention to the management of the contralateral breast. In this chapter the authors will discuss and provide a systematic method to correctly plan and approach this kind of surgery.

38.2 Preoperative Evaluation

Preoperative evaluation is fundamental in order to choose the appropriate surgical technique for remodeling the contralateral breast after breast oncological procedure.

In order to obtain the best symmetry between the two breasts in terms of volume and shape, several features should be considered:

1. Type of oncologic resection.
2. NAC position: any asymmetry between the two NAC must be assessed.
3. Mammary ptosis: true ptosis or pseudoptosis must be discerned; the degree of glandular descent and of skin exceeding and stretching must be evaluated and distinguished in planning the correct mastopexy or reduction mammoplasty technique, when needed.
4. Volume: any difference and asymmetry in mammary volume must be assessed between the two breasts. If the affected breast is bigger than the contralateral one.
5. Breast shape: in particular stenotic and tuberous breast deformities should be recognized and assessed.
6. Symmetry.
7. NAC-inframammary fold distance.
8. Alignment of the NAC along the breast meridian. The NAC can be displaced both laterally and medially.
9. Thickness of the patient's skin. In patients with excessively thin skin, the breast implant contours may be easily visible giving an unpleasant and artificial appearance to the breast and leading to the loss of the natural harmonic breast profile.
10. Thoracic deformity deriving from a variety of conditions as prominent costal bones, sternal anatomical deformities or alterations, and scoliosis.
11. Patient's requests.

1. *Preoperative pictures:* pictures in six projections are made before surgery, as shown in images. To assess the precise position of the mammary crease, the patient is also asked to lift her arms above her head.
2. *Preoperative marking* is made requiring the patient to stand in front of the surgeon, with arms hanging relaxed along the body. A vertical median line is drawn running from the interclavicular junction to the umbilicus. The jugulum to NAC distance is then measured and compared between the two breasts. The areolar perimeter and the inframammary folds are marked. The breast meridians are finally drawn along with the base of the breasts. Mastopexy and reduction mammoplasty markings are modified during surgery when immediate reconstruction is performed, since the

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contralateral breast will be adjusted to the diseased one after its reconstruction. The seatback of the operating table is lifted up to 45° during surgery in order to recreate the gravity effect and correctly adjust the markings. When a two-step reconstruction is performed, markings are made preoperatively, since the final volume and shape of the reconstructed breast are assessable previous to surgery.

The preoperative meeting with the patient is extremely important. During the encounter the goals of the surgical reconstructive procedure are clarified and explained: even if the main aim is to obtain symmetry between the two breasts, the physician must underline that equality between them cannot be guaranteed.

Patients' comorbidities and previous or current therapies must be considered too. Patients with a positive history for neoadjuvant chemotherapy or radiotherapy have an increased surgical risk for local infections and flap necrosis.

In order to correctly consider the entity of the oncologic resection planned for the diseased breast and consequently to correctly choose the technique to apply to the contralateral breast, a multidisciplinary approach is advocated. Thus the patient is evaluated together with the general surgeon previous to surgery.

Further preoperative and intraoperative considerations regarding the different techniques and situations will be discussed in the next sections.

38.3 Surgical Techniques

Depending on the oncological surgical technique employed on the affected breast, different reconstructive surgical techniques can be adopted to remodel the contralateral breast in order to symmetrize and adapt it to the former. A list of the employable techniques is here provided. In the next section, the authors will discuss in which case each technique is suitable and suggested.

38.3.1 Mastopexy

Mastopexy literarily means lifting up of the breast. Classically mastopexy is used to correct breast ptosis, which in turn can be due to a glandular ptosis, skin stretching and excess, or both. Actually by the employment of this technique, not only a lifting of the breast can be achieved but also a replacement of the NAC. Depending on the degree of breast ptosis, amount of breast tissue, and amount of skin excess, three main different mastopexy techniques can be used:

- Periareolar mastopexy: this technique can be used to correct low to medium degrees of mammary skin ptosis. In addition to the correction of a mammary ptosis, the goal in performing this technique is to centralize the NAC above the point of maximum projection of the breast mound and/or symmetrize it to the contralateral when the two NAC are not symmetrically positioned [1]. According to this the markings and consequently the incisions may be eccentric rather than concentric (Fig. 38.1). When a medial NAC repositioning is wanted, the markings and incisions will be elliptical having the major axis of the ellipse horizontal and having the lateral margin of the NAC corresponding to the lateral margin of the ellipse. The longer the major axis of the ellipse, the more the NAC will result medial in respect to the original position. The same method is adopted in order to lateralize, lower, or lift the NAC, accordingly

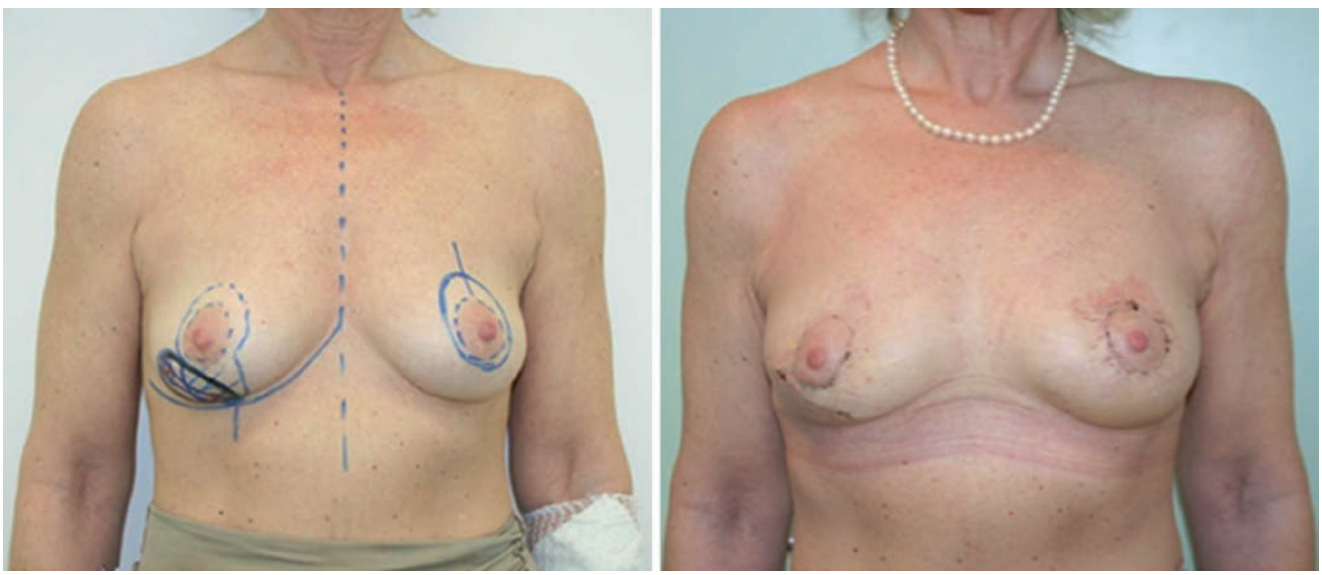

Fig. 38.1 Right breast inferolateral quadrantectomy. Periareolar vertically eccentric mastopexy is planned for contralateral breast management

changing the orientation and major axis of the ellipse. The wider the ellipse drawn, the more the amount of the skin that will be excided. Consequently the size of the ellipse should be increased along with the degree of skin excess.

The surgical incisions will follow the markings made in the preoperative evaluation. After the periareolar skin incision has been done, a second external cutaneous incision is made following the markings. The skin comprehended by the two incisions is then deepithelized. Consequently the dermal layer is incised circumferentially except for a small portion in the cranial part which will function as superior dermal pedicle. Dermal round-block sutures allow to reduce the diameter of the external incisions, thus reducing the skin tension affecting the eventual periareolar sutures. Reabsorbable Vicryl sutures are employed for the gland, and reabsorbable Monocryl intradermic sutures are used for the dermal layer.

- Vertical and inverted T mastopexy
  For medium to high and high degrees of mammary skin ptosis with breast volume and for glandular ptosis, an inverted T approach must be employed.

  Since the markings and techniques employed in performing a vertical or inverted T mastopexy are nearly the same of those employed in performing a vertical or inverted T reduction mammoplasty, these two approaches will be discussed and described in the reduction mammoplasty section.

In order to choose the correct mastopexy technique, the quality of mammary ptosis must be correctly assessed. When a true glandular ptosis is present and the mammary gland is majorly distributed at the lower quadrants, a reversed T mastopexy is usually advocated, allowing to reposition the gland upward and to remove the excess of glandular tissue. When the ptosis is purely glandular, repositioning of the NAC may not be necessary. When the ptosis is mainly due to a skin excess, a periareolar or vertical approach may be sufficient. In this latter case, a descent of the NAC secondary to skin stretching and descent is usually present and its repositioning is needed.

38.3.2 Reduction Mammoplasty

Reduction mammoplasty is employed to correct an excess in glandular volume. Since a variable degree of mammary ptosis and skin excess is always observed in hypertrophic mammary glands, lifting of the gland and excision of exceeding skin are also performed along with the excision of the exceeding glandular tissue. Three main different approaches are employed in reduction mammoplasty: periareolar, vertical, and inverted T approach:

- Periareolar reduction: surgical approach and markings are the same as described in the periareolar mastopexy section. By the use of this technique, small to medium volume reductions can be achieved. As a consequence this approach should be chosen when a smooth volume asymmetry is noted between the two breasts, after cancer excision and affected breast reconstruction. When quadrantectomy is performed, glandular reduction of the contralateral breast can involve the same quadrants as the ones resected in the affected breast or different ones, depending on the patient's preoperative anatomy. Volume symmetrization through this approach can be adopted also after mastectomy and reconstruction with similar indications obtaining good results with minimal scarring (Fig. 38.2).
- Vertical reduction mammoplasty: this technique can be employed when medium degree of glandular hypertrophy or exceeding is observed. Periareolar markings are first made. Secondly, the desired new position of the NAC is assessed: by holding the breast between the thumb and the third finger, the projection of the midpoint of the inframammary fold on the anterior surface of the breast can be marked; this point will indicatively correspond to the cranial margin of the NAC. The markings are consequently made. The two vertical lines connect the extremities of the circle to the midpoint of the inframammary fold. Indicatively the wider the vertical lines, the more will be the skin and glandular excision. The shape of the circle or ellipse allows to modify the quantity of the skin excised in the central and cranial part of the gland and allows to modify the position of the NAC in respect of the mammary mold, similarly to what has been previously described in the periareolar mastopexy section. The cutaneous incisions follow the markings described. The gland is also incised accordingly to the markings. A vascular pedicle cranially to the NAC is maintained and no glandular incision should be made in this region. A variable amount of glandular tissue can be excised in order to reduce the mammary volume. The amount of the skin excised not necessarily corresponds to the amount of gland removed: by increasing the depth of the glandular incision, increased amounts of glandular tissue can be removed. Dermoglandular detachment is usually performed along the margins of the incisions in order to avoid excessive tension on the suture lines. The dermoglandular flaps are eventually approached and sutured [2, 3].
- Inverted T reduction mammoplasty: this technique is usually employed when a medium to high degree of hypertrophy or mammary ptosis is observed. The length of the vertical lines is approximately 5–6 cm. This will be the final length of the NAC-inframammary fold distance. Depending on the degree of ptosis and hypertrophy of the mammary gland observed preoperatively, an upper or

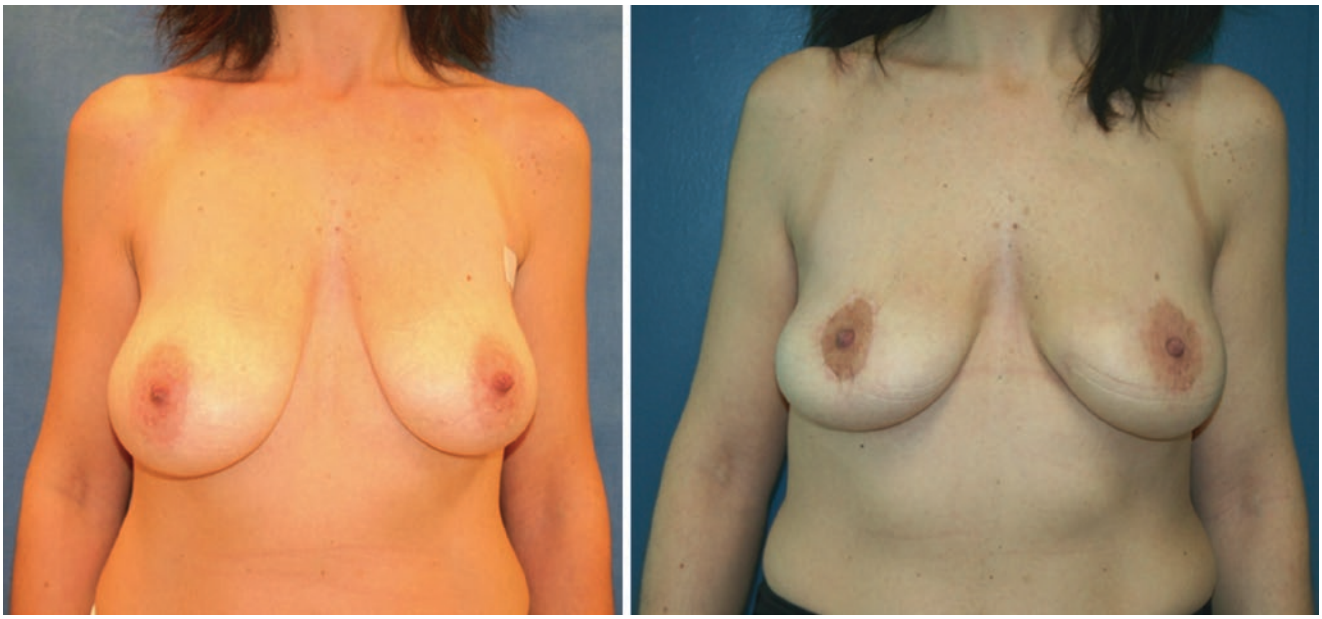

Fig. 38.2 Left breast inferior quadrantectomy. Periareolar vertically eccentric reduction mammoplasty has been performed for contralateral breast volume symmetrization. Breast parenchyma has been removed in the lower quadrants of the right breast in order to balance volume asymmetry, already present before surgery, with minimal scarring

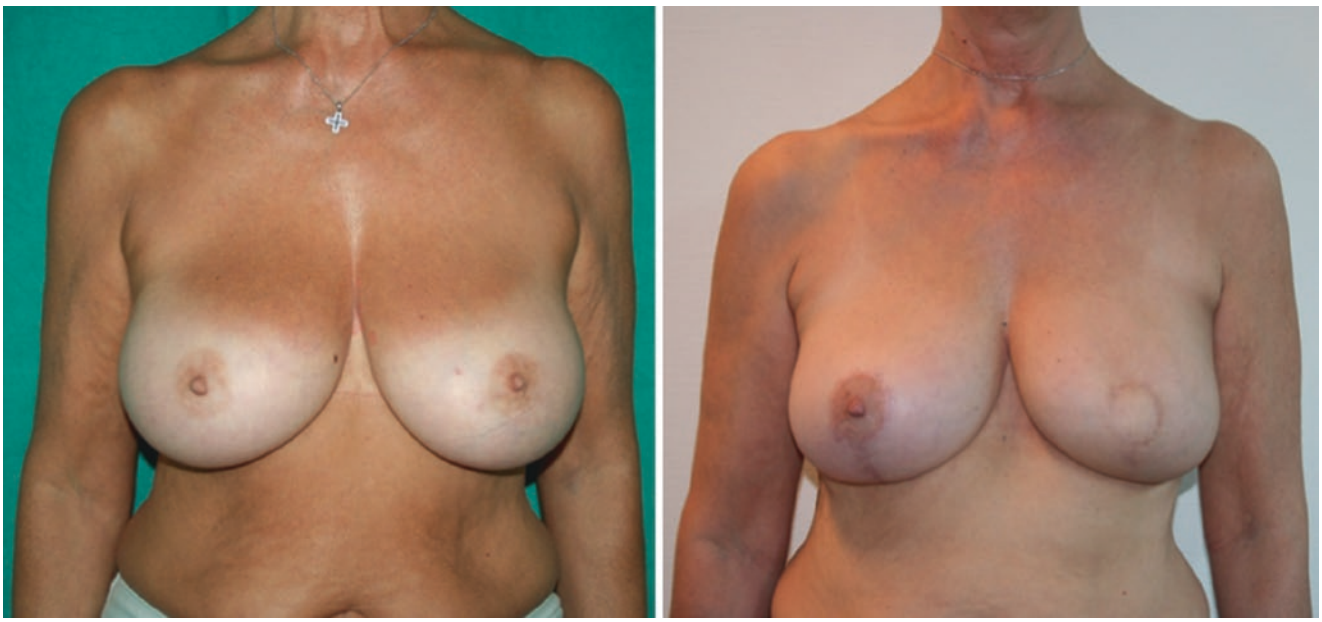

Fig. 38.3 Left breast central quadrantectomy. Inverted T reduction mammoplasty that is adopted has been chosen for contralateral breast volume symmetrization. A lower pedicle is adopted with a glandular excision in the lower quadrants

lower vascular pedicle is chosen. A lower pedicle is usually employed when a severe hypertrophy and degree of ptosis are present. In fact an upper pedicle in these types of breast would be excessively long and would need to be folded several times to allow the repositioning of the NAC cranially, thus increasing the risk of vascular necrosis of the NAC itself. The glandular excision involves mainly the lower quadrants. Comparing to the vertical technique, a wider amount of gland is removed. The glandular tissue remaining after the excision is usually rearranged and lifted: a detachment of the mammary gland from the muscular fascia allows to lift up the glandular tissue and to suspend it cranially by suturing it to the pectoral fascia (Fig. 38.3).

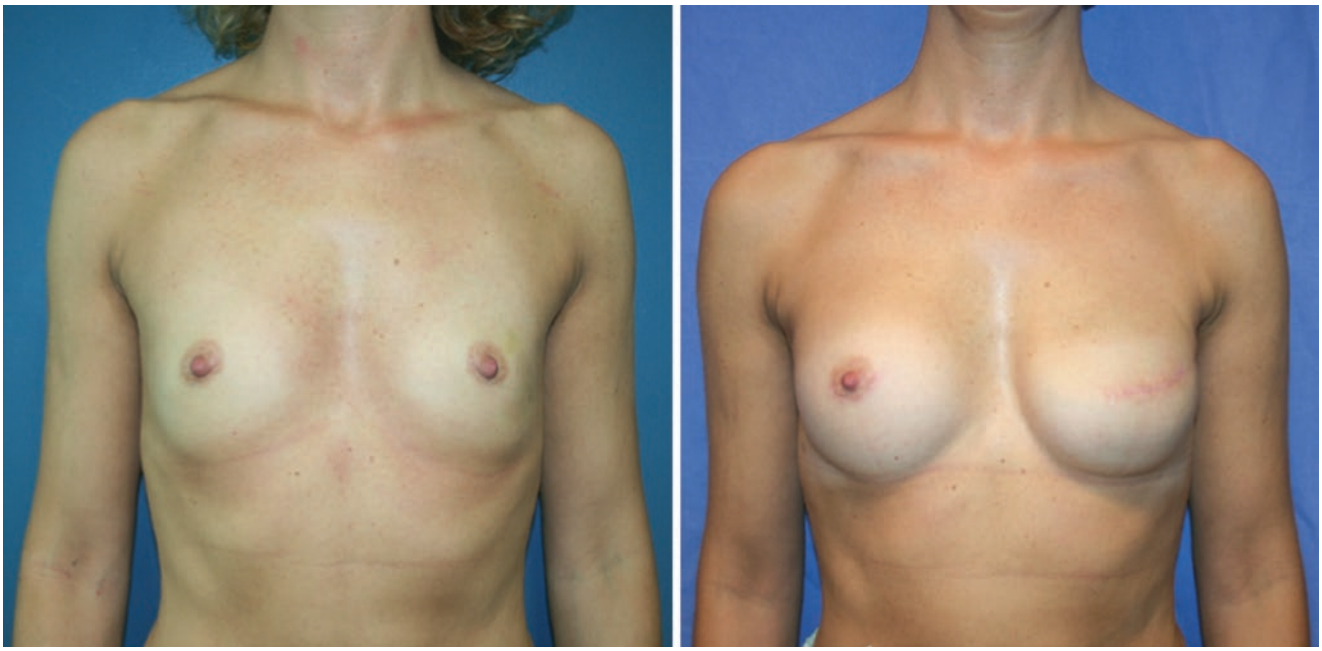

Fig. 38.4 Left breast mastectomy and immediate two-stage breast reconstruction. During stage II breast reconstruction, contralateral symmetrization has been performed with augmentation mammoplasty since unaffected breast was hypoplastic

When a residual excess of dermoglandular tissue is still present alongside the inframammary fold, a further dermoglandular excision can be performed. When an increase in breast projection is aimed, a portion of the lower quadrants' glandular tissue can be undermined and preserved: by flipping inward the resulting glandular flaps (autoprosthesis), the projection of the breast can be significantly increased [4–14].

38.3.3 Augmentation Mammoplasty

Augmentation mammoplasty assumes the use of breast prostheses. The cutaneous incisions can be periareolar or performed at the inframammary fold or axilla. The authors prefer the periareolar approach for several reasons. Firstly, when a periareolar mastopexy is necessary, the same surgical access can be employed for both techniques. Secondly, the scar can be camouflaged corresponding to the limit between the areolar darker skin and the breast lighter skin. Moreover when a modification or definition of the inframammary fold is needed, the periareolar surgical access allows to work on it more comfortably when compared to the inframammary surgical access. The prostheses can be implanted both in the subglandular plane or in the submuscular plane. If no anatomical abnormalities are found and the pectoralis major is normotrophic and integer, a submuscular approach is preferred. The dual plane technique is usually adopted: the pectoralis major muscle is interrupted at two thirds of its length parallel to the muscular fiber to avoid excessive bleeding and postoperative pain. When anatomical conditions are not optimal or the pectoralis muscle is hypotrophic or atrophic, a subglandular approach is preferred. In both the submuscular and subglandular techniques, the pocket is created by digital dissection in order to avoid excessive traumatism to nerves and vessels. The ideal pocket should be large enough to host the new implant without distorting it, being careful not to exceed cranially and medially which would lead, respectively, to riding high and symmastia deformities. A new inframammary crease is needed when the contralateral one is asymmetrical or if the previous mammary fold is aesthetically unpleasant. The new inframammary crease is defined by suturing the lower pole dermal and subcutaneous tissue to the costal perichondrium or to the caudal capsular tissue. Failure in performing this may lead to bottoming out or double-bubble deformities. The pocket is closed with interrupted sutures. A drain is always inserted in the pocket (Fig. 38.4).

38.3.3.1 Contralateral Breast Management in Case of Quadrantectomy

Quadrantectomy means the excision and removal of a part of the mammary gland. The volume and position of the glandular mass removed vary depending on the volume and the position of the tumor.

Quadrantectomy inevitably causes a reduction in the mammary volume of the affected breast leading to an

unavoidable asymmetry between the two breasts, if they are initially equal. Depending on the amount of tumorous and glandular tissue removed, the resulting asymmetry degree will accordingly vary. The location of the cancer and the affected quadrants do not importantly affect the approach on the contralateral breast, being the main variable counting the amount of tissue removed. The breast initial volume also influences the technique chosen to reconstruct both the affected and the contralateral breast.

When the patient's breasts are equal in volume or when the affected breast is smaller than the contralateral one, three main situations can be discerned:

1. A small amount of volume is removed from the affected breast. In this case no intervention on the contralateral breast may be needed. If an asymmetry derives from the excision of the tumor, a periareolar mastopexy is usually sufficient to symmetrize the contralateral breast and NAC.
2. A large amount of volume is removed from the affected breast and the patient's breast is normotrophic or hypertrophic. In this case a reduction mammoplasty and mastopexy are usually employed. If the resulting asymmetry is smooth, a periareolar approach may be sufficient for parenchyma reduction, while when asymmetry is more severe, a vertical or reverse T approach may be needed.
3. A large amount of volume is removed from the affected breast and the patient's breast is hypotrophic. In this case simply remodeling and reshaping the remaining volume on the affected breast do not allow a satisfactory reconstructive result, and a prosthesis implantation is needed to give the affected breast an acceptable morphology. If the affected reconstructed breast appears bigger than the contralateral one, a prosthesis implantation may be needed also in the unaffected side. The prosthesis chosen for the contralateral breast will be smaller than the one employed to reconstruct the diseased breast, since the glandular tissue is entirely preserved in the former. A circumareolar mastopexy is usually employed if a certain degree of ptosis is observed along with breast hypotrophy and in order to symmetrize the NAC.

When the affected breast is bigger than the contralateral one, no intervention on the latter may be needed: the reduction of the diseased breast derived by the quadrantectomy may adjust itself the affected breast to the healthy one. A simple periareolar mastopexy may be needed to symmetrize and reposition the NAC. If a large amount of volume is removed from the affected breast, the three previously reported situations are possible.

When radiotherapy consequent to surgery is necessary and programmed, any intervention on the unaffected breast should be postponed 3–6 months after the end of the radiotherapy cycles. Tissues undergoing radiotherapy may in fact suffer from chronic edema or fibrosis. In the first case, the breast will appear more and more swollen over time; in the latter it will progressively reduce. Thus modifying and adjusting the contralateral breast previous to radiotherapy may result useless and unsuccessful. Only when a great difference is observed between the two breasts after quadrantectomy, surgery is suggested on the contralateral gland.

When breast implants are adopted in reconstructive surgery by the insertion of a prosthesis in the breast undergoing quadrantectomy without a contralateral augmentation mammoplasty for symmetrization, a particular attention should be given. In such cases the aging processes will differ between the two breasts: the breast reconstructed by the implantation of a prosthesis will minimally change over time, while the contralateral one will vary in size, accordingly to the possible weight gain or loss of the patient, and will descend over time. Consequently asymmetry between the two breasts may turn out again over years after reconstruction and further surgery may be needed. The patient must be aware of this aspect, and the physician must clearly explain it at the preoperative encounter and consultation.

38.3.3.2 Contralateral Breast Management in Case of Mastectomy

Mastectomy means the removal of the entire mammary gland in the presence of a tumor. Depending on the different types of mastectomies and on the anatomical characteristics of the patient, two main reconstructive routes can be followed:

- Immediate reconstruction: the affected breast is reconstructed immediately after the oncologic resection by the use of a prosthesis or flaps.
- Two-stage reconstruction: the affected breast's volume is recovered over time by progressive expansions of an expander inserted at the time of the oncological resection. When the expansions are terminated, the expander is eventually replaced by a definitive mammary prosthesis.

When an immediate reconstruction is planned, contralateral breast reconstruction is made at the same time of the affected breast reconstruction. Since a portion of the skin may be removed together with the gland from the diseased breast, it may result smaller and lifted after the reconstruction. Thus a mastopexy and reduction mammoplasty are usually needed for the contralateral breast. Depending on the volume, degree of ptosis, and morphology of the unaffected breast, all the different mastopexy or reduction mammoplasty techniques previously described may be employed. If radiotherapy is to be performed on the affected breast, the reconstruction of the contralateral one should be postponed

3–6 months after the end of the last radiotherapy cycle, in order to appreciate any possible breast swelling or volume reduction.

When a two-stage reconstruction is planned, contralateral breast reconstruction is made at the time of the substitution of the expander with the definitive breast prosthesis. In fact the eventual appearance of the diseased reconstructed breast is not predictable at the time of the expander insertion. If radiotherapy is to be performed, the second stage of the reconstruction should be postponed 3–6 months after the end of the last radiotherapy cycle, in order to appreciate any possible breast swelling or volume reduction in the affected breast, as mentioned in the previous paragraphs. The possible situations at the time of a second stage reconstruction are listed as follows:

- *The contralateral breast has a major degree of ptosis and/or is greater in volume compared to the reconstructed breast:* this represents the commonest situation. Patients undergoing mastectomy are adult women, most of which over 40 years old. Consequently a certain degree of ptosis is generally present. Moreover when a mastectomy is performed, a variable amount of the skin may be excised along with the gland, as described before. As a result the contralateral breast usually proves to have a major volume when compared to the reconstructed one, even after the expansions have been completed. Depending on the severity of mammary ptosis and of the breast hypertrophy, different mastopexy and reduction mammoplasty techniques may be adopted, allowing to reduce and lift up the mammary gland, to excise the possible excess of the skin, and to reposition the NAC correctly.
- *The contralateral breast presents the same degree of ptosis and the same volume of the reconstructed breast:* in these rare situations, no intervention is needed on the contralateral breast. A periareolar mastopexy for minimal asymmetry between the glands or the NAC position may be performed.
- *The contralateral breast is smaller in volume compared to the reconstructed breast:* this may happen especially when a skin- or nipple-sparing mastectomy is performed, allowing a major expansion volume. The contralateral breast may be hypotrophic or atrophic. It may be or not be descended. This represents an extremely rare circumstance. In such case the implant of a prosthesis in the contralateral breast, along with a mastopexy (if necessary), is needed. As described in the quadrantectomy section, the prosthesis employed will be smaller than the one used to reconstruct the affected breast, since the mammary gland is spared in the contralateral breast and contributes to its final volume.

The previous consideration about aging processes in contralateral breast should be made for patients undergoing mastectomy breast implant reconstruction without a contralateral augmentation mammoplasty for symmetrization. In fact in these cases, which are the more frequent, the two breasts will differ over time. The breast reconstructed by the implantation of a prosthesis will minimally change over time, while the contralateral one will vary in size, accordingly to the possible weight gain or loss of the patient, and will descend over time. Consequently asymmetry between the two breasts may turn out again over years after reconstruction and further surgery may be needed. The patient must be aware of this aspect, and the physician must clearly explain it at the preoperative encounter and consultation.

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Lipofilling

39

J.Y. Petit, V. Lohsiriwat, and M. Rietjens

Although lipotransfer is not a new technique [1], it can be considered a technical revolution in plastic surgery which is widely performed all over the world for aesthetic surgery [2]. More recently, the lipofilling has been indicated in breast cancer patients to improve the results of breast reconstructions, and [3–6] current literature underlines the efficacy of the technique as well as the safety of the procedure in cancer patients. Applications of lipofilling have been performed to improve the shape of breast reconstruction with prosthesis or with autologous musculocutaneous flaps. Fat injection can also be used to reshape the bad results of the conservative treatment. Most of the defects observed after conservative treatment can be easily filled up with fat tissue instead of glandular or distant flaps. Different teams are now investigating the possibility of refilling the defect of the quadrantectomy immediately at the time of the quadrantectomy. Total breast reconstruction with pure fat refilling is also performed as demonstrated in several studies [7, 8].

39.1 Application of Lipofilling in Breast Cancer Surgery

Lipofilling is being indicated for soft tissue defect correction in many sites. It is not only for corrective surgery but also for cosmetic purpose. For breast cancer surgery, lipofilling procedure might be proposed in the following situations:

- Correction of defects and asymmetry following wide local excision (or breast conservative surgery), with or without radiotherapy
- Improvement of soft tissue coverage following implant-based breast reconstruction
- Volume replacement of implants in unsatisfactory oncoplastic breast reconstruction outcomes
- Augmentation of volume and refinement after autologous breast reconstruction
- Whole breast reconstruction with serial fat grafting
- Scar correction

39.2 Technique of Lipofilling

Lipofilling can be performed under general or local anesthesia. Generally, the aim of the technique is to decrease cell damage and to promote survival of the fat tissue and its composition. Success is heavily dependent on the technique used for harvesting, preparing, and grafting of the fat (Figs. 39.1, 39.2, 39.3, 39.4, 39.5, 39.6, 39.7, 39.8 and 39.9).

39.2.1 Identification of the Donor Site

The most common site is the abdominal fat because it is one of the most fat deposit area. Moreover, there is no need to change the patient's position in the operation room. The second site is the trochanteric region (saddle bags) and the inside of the thighs and knees. The harvesting areas are outlined with a skin marker. But every location showing an excess of fat tissue on the body can be used for fat liposuction.

39.2.2 Preparation of the Solution

The tumescent solution (so-called Klein's solution) is prepared to be injected into the donor site: 1 cc of epinephrine (1:500,000) diluted in 500 cc of 0.001% lactated Ringer's solution (LRS). The 50 cc of mepivacaine can be added in

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Fig. 39.1 Lipofilling technique: abdominal liposuction, centrifugation, purification of the fat, reinjection in the breast

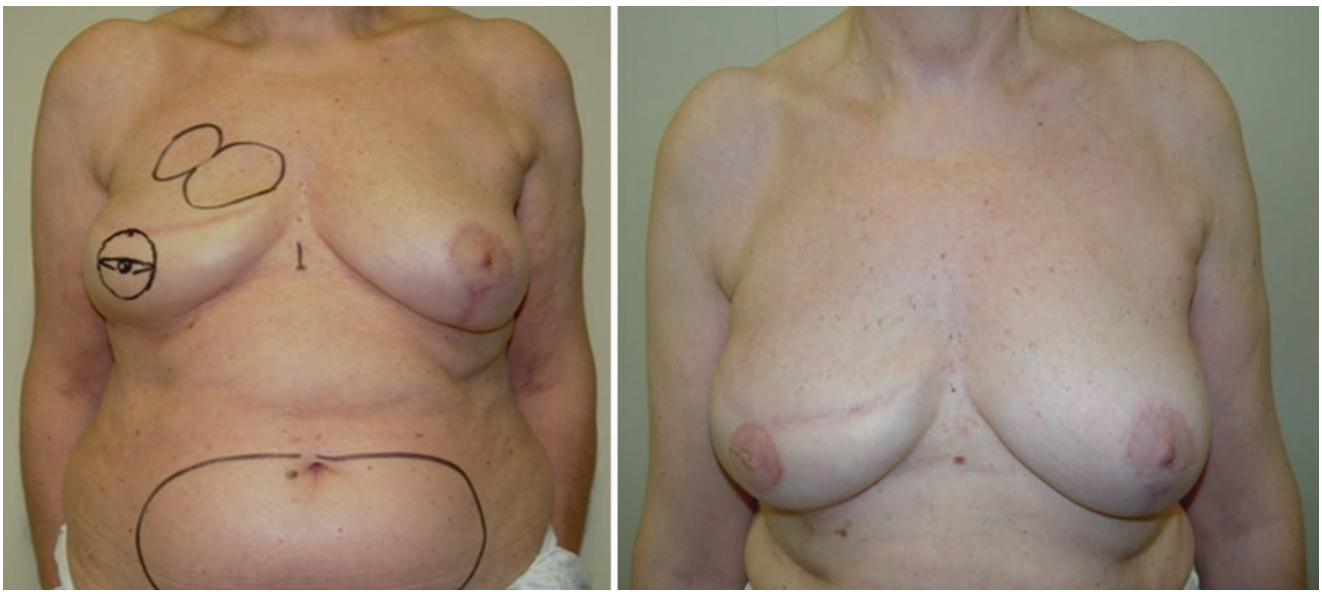

Fig. 39.2 Improvement of the *right* implant breast reconstruction with lipofilling

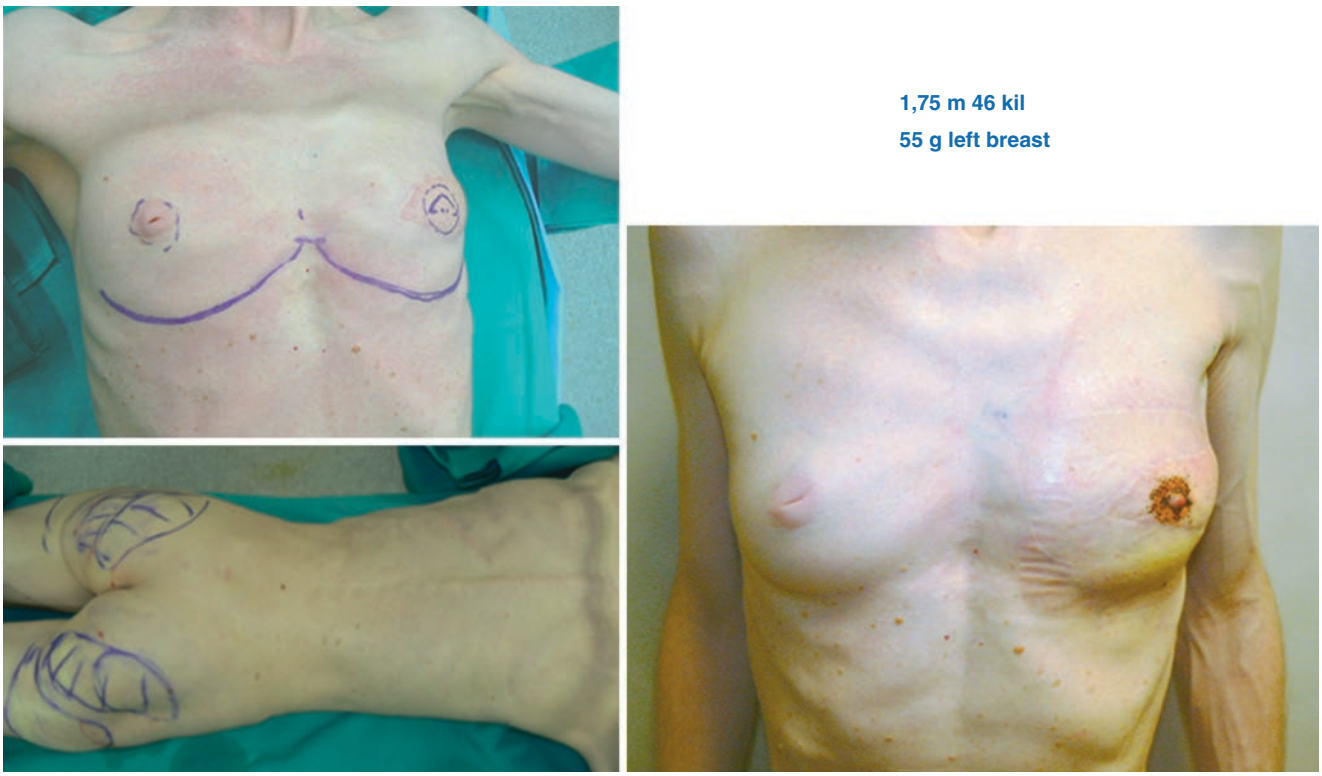

Fig. 39.3 Improvement of the *left* prosthetic breast reconstruction with lipofilling in a very thin patient

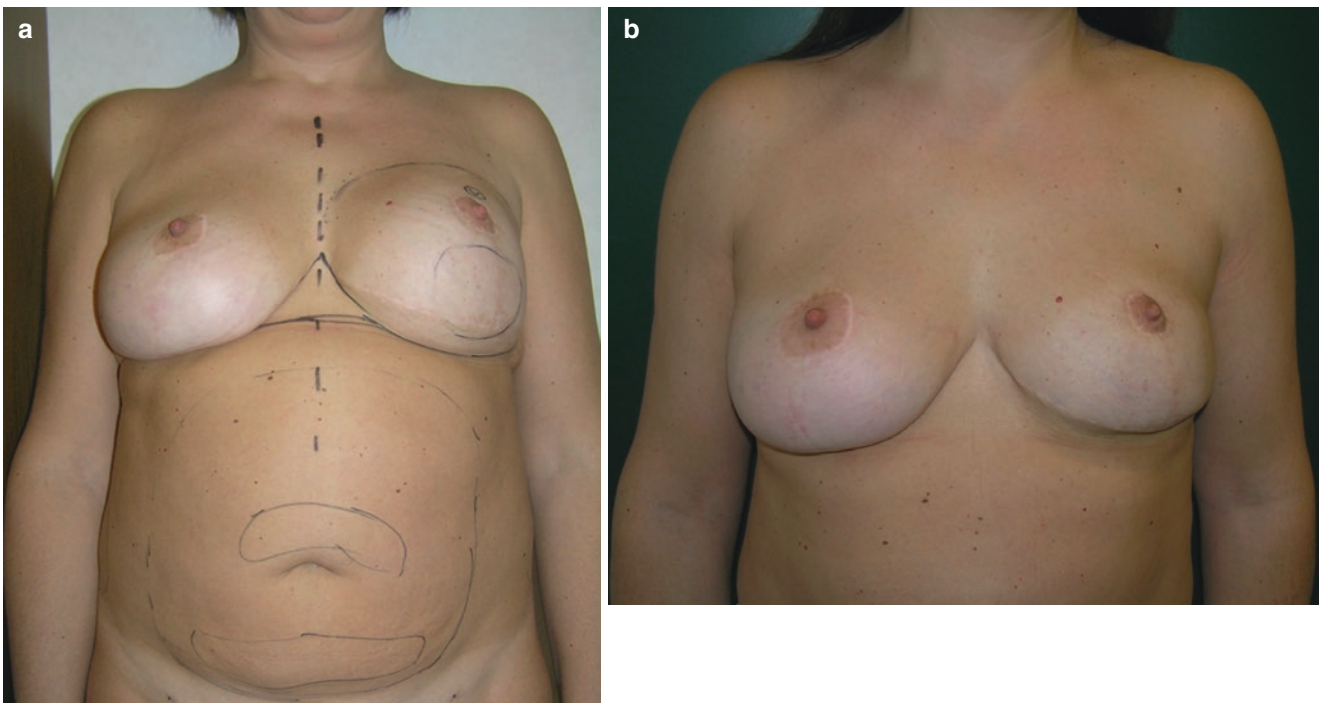

Fig. 39.4 Total *left* breast reconstruction with pure lipofilling

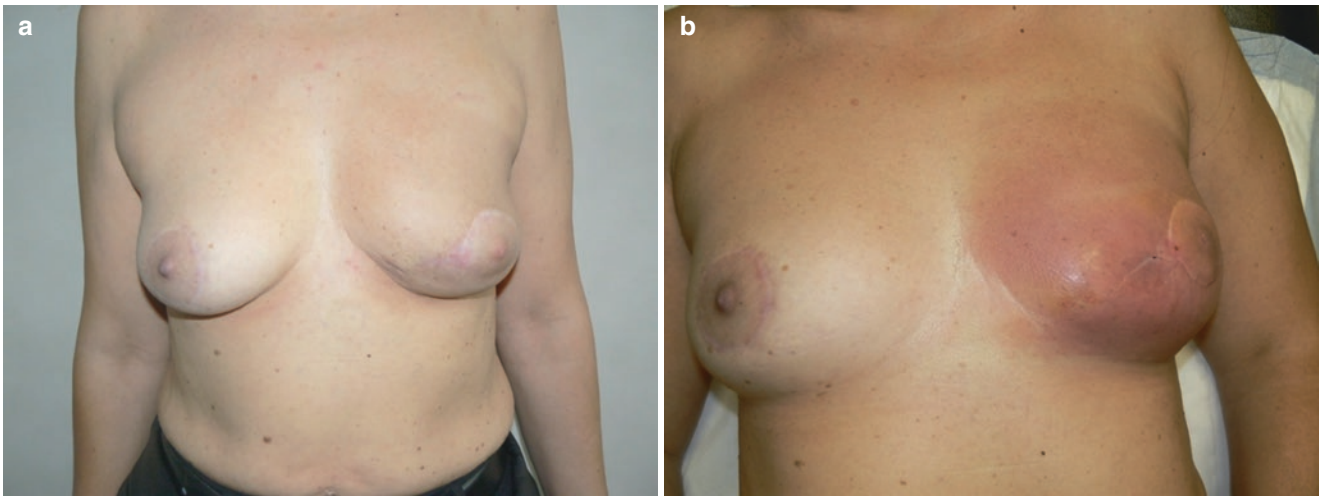

Fig. 39.5 Infection and result after drainage and antibiotherapy

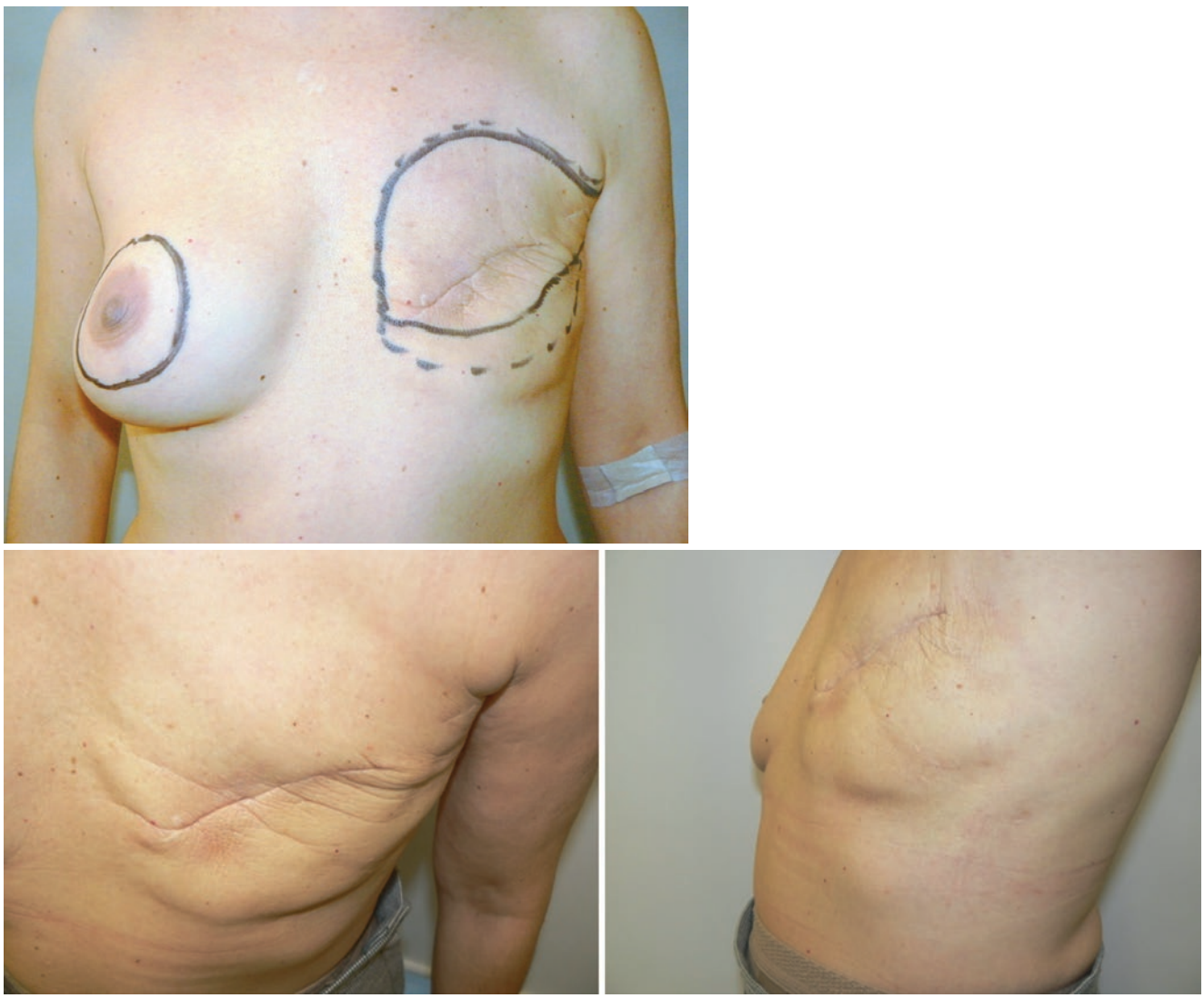

Fig. 39.6 Improvement of the mastectomy scar after previous radiotherapy and before implant breast reconstruction

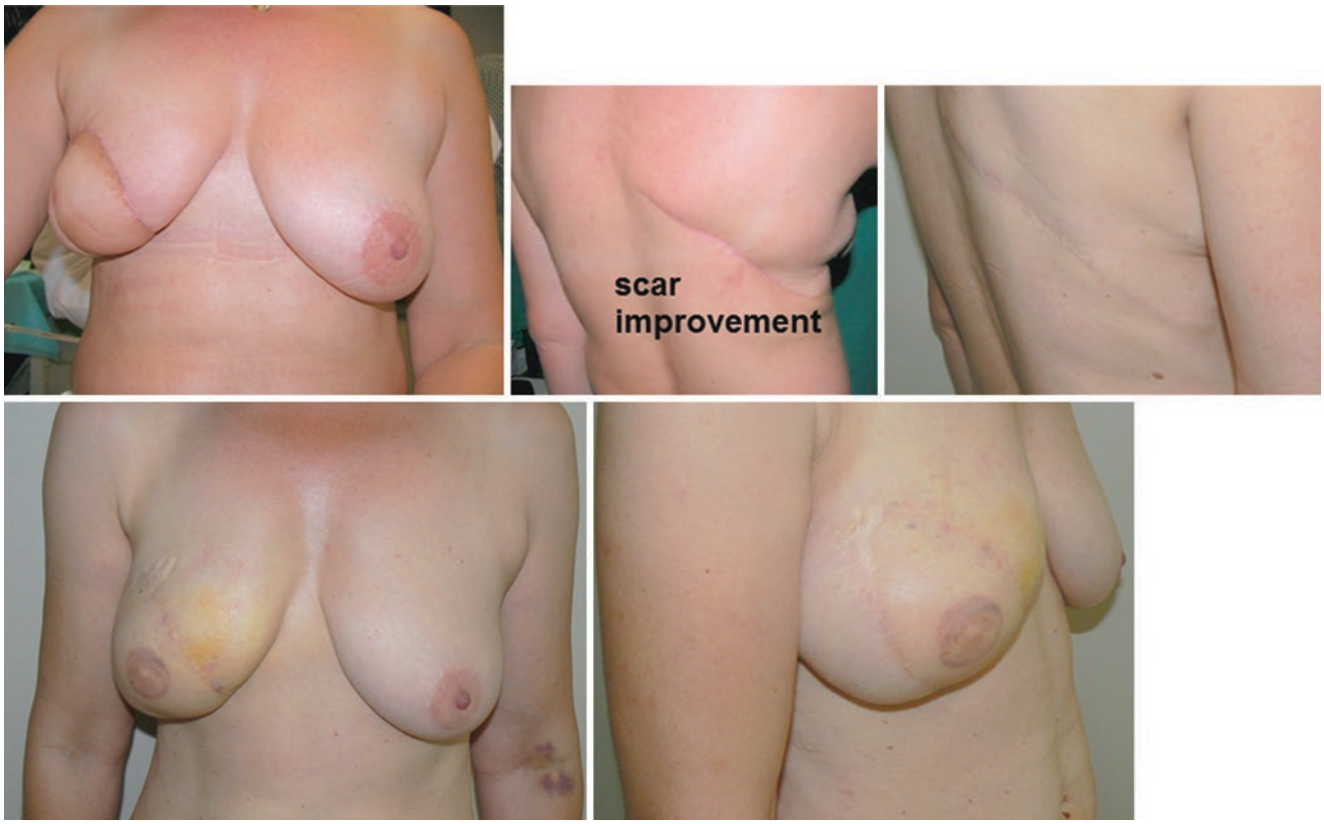

Fig. 39.7 Improvement of autologous latissimus breast reconstruction and improvement of the dorsal scar

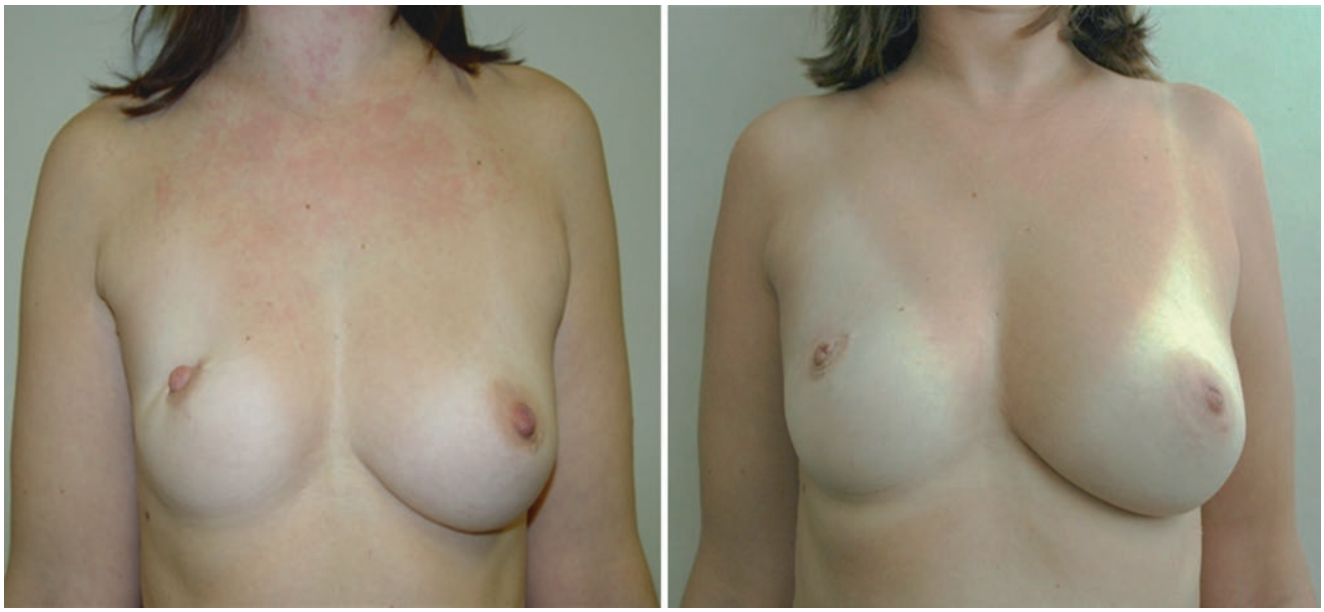

Fig. 39.8 Improvement of BCT with lipofilling

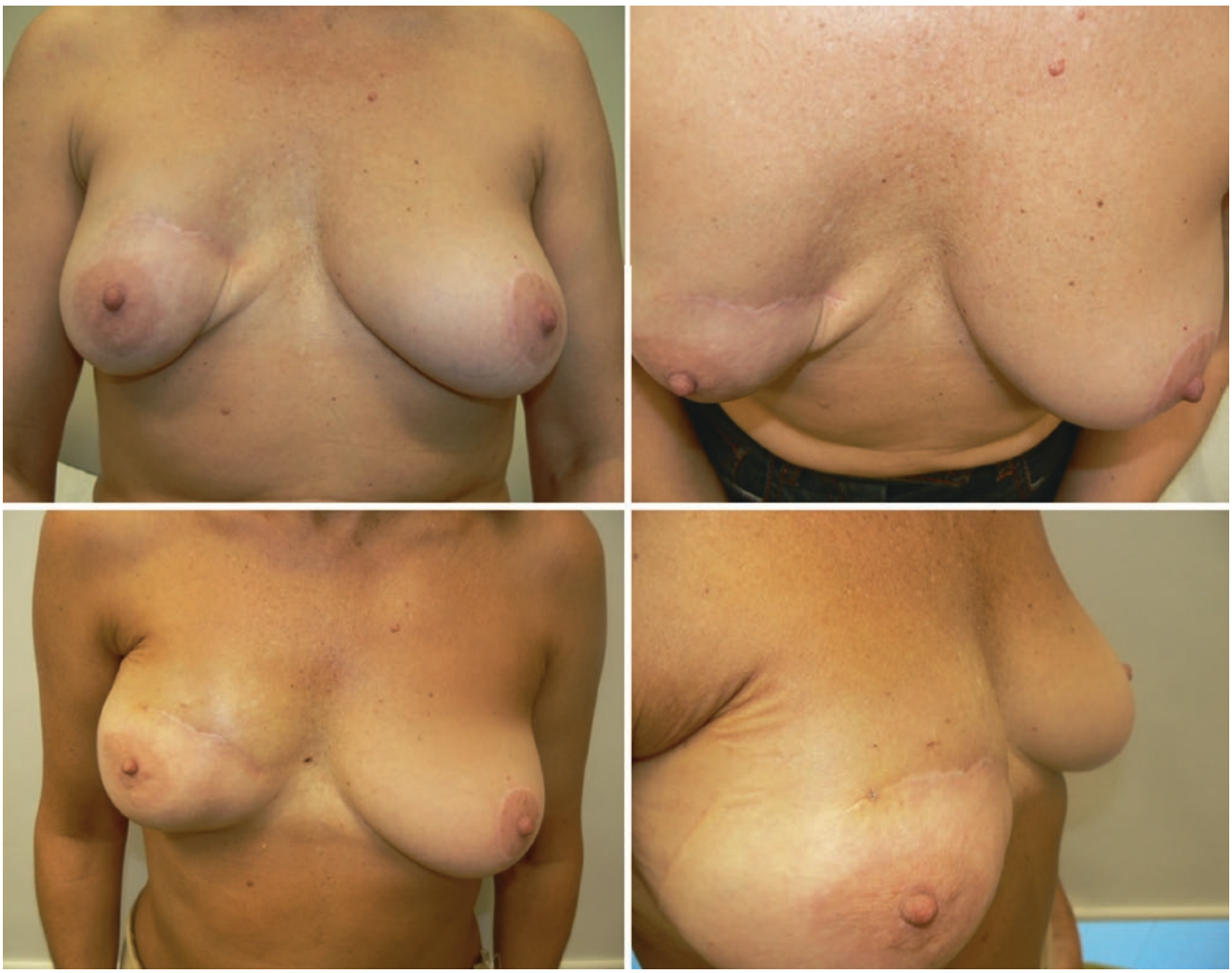

Fig. 39.9 Reshaping of the *right* breast conservative treatment with lipofilling

the solution if the procedure is planned under local anesthesia. It is injected through a small-bore 4 mm blunt cannula that was attached to a 60-cc syringe. The estimate volume of solution is 1 cc for each 1 cm^3 of target fat harvest volume. The surgeon should wait at least 15 min before starting fat harvesting; the adrenaline is added to the solution in order to achieve well hemostasis and to decrease postoperative pain.

39.2.3 Fat Harvesting

The most well-known technique of fat harvesting has been described by Coleman [9]. The procedure starts through a small incision made in the abdomen by blade no.11 and gradually applies a blunt tip harvesting cannula (3 mm in diameter and 15 or 23 cm in length). Manually, the syringe is drawn to create to low negative pressure during fat harvesting. The cannula is attached to 10-cc Luer Lock syringes. However, various techniques of fat harvesting with different cannula or liposuction machine system have been reported with different outcome assessments.

An "experimental solution" study by Ozsoy et al. demonstrated a greater number of viable adipocytes when harvested with a 4-mm-diameter cannula compared with 2- or 3-mm cannulas. Erdim et al. also recommended the use of larger cannulas to increase cell viability. Their study showed more viability of fat cells when using 6-mm-diameter cannula than using 2 or 4 mm in diameter cannula.

Different vacuum pressures and some assisted techniques have been used in many clinical series. Rohrich et al. compared traditional liposuction, internal ultrasound-assisted liposuction, external ultrasound-assisted liposuction, and massage-assisted technique liposuction. There was no significant histologic or chemical effect of external ultrasound-assisted liposuction on harvested adipocytes. Pu et al.

compared the Coleman technique versus conventional liposuction technique and found significantly higher viable adipocyte level in the Coleman technique. Crawford et al. examined the hand aspirate at low-force centrifuge versus standard power-assisted liposuction and showed higher cell counts which were observed when using the low-force centrifuge [10–20].

39.2.4 Fat Processing

The most frequently used methods for fat processing are centrifugation, washing, and decantation. The purified fat can be separated from cell debris by centrifugation, as described in the widely used protocol by Coleman. After centrifugation, the lipoaspirated specimen can be separated into four layers: (I) the oily fraction, leaked out of disrupted adipocytes; (II) the watery fraction consisting of blood, lidocaine, and saline, injected before the liposuction; (III) a cell pellet on the bottom; and (IV) the purified fat between the oily and the watery fractions. For washing technique, the fat is washed using normal saline or 5% glucose solution in order to wash out the blood and the oil part and cellular debris from the aspirated fat. The least popular technique is decantation, which uses the gravity effect to precipitate the cellular component from the oily and water component.

39.2.5 Fat Transfer (Injection)

At EIO, we are using modified Coleman technique by injecting the processed fat via 2-mm-diameter cannula attached to 1 mL to 3 or 10 mL disposable syringe. The fat is transferred directly to the breast, trying to avoid intraparenchymal injection and avoid creating the bolus injection. The entry site of the cannula can be made by sharp blade or a sharp 17-gauge needle to minimize the scar. It is mandatory to overcorrect the defects because 40–60% of the transferred fat is expected to be resorbed. Experimental studies have found that up to 90% of transplanted adipose tissue could be lost, while clinically reported figures range between 40% and 60%, and most of the volume loss occurs within the first 4–6 months following surgery. Despite, several novel techniques proclaimed that they produced more effective outcomes. Nonetheless, surgeon should calculate the quantity of fat preparation and injection before the procedure. The limitation of volume inject can be due to the recipient tissue quality such as in irradiation tissue or thick scar. If the target volume cannot be achieved by a single lipofilling procedure, then the patient should be informed for the possibility of repeated lipofilling. In general the overcorrection reaches an excess of around 40% of the amount of fat required to correct the defect.

39.2.6 Lipofilling in Reconstruction of Irradiated Breast

External radiation is required in breast conservative treatment and after total mastectomy in case of positive nodes. Salgarello et al. retrospectively studied 16 patients who underwent lipofilling to the chest wall after irradiation and then followed by prosthesis introduction and found high success rate of prosthetic-based procedure with no complication or oncological recurrence [21]. Sarfati et al. performed lipofilling prior to implant introduction after irradiation in 28 patients. They reported high success rate with only one prosthesis exchange due to prosthesis exposure after lipofilling [22]. Rigotti also demonstrated the efficacy of lipotransfer to improve the radiodystrophic sequelae [23].

39.3 Complication

39.3.1 Recipient Site Complications [23, 24]

- Fat necrosis, oil cyst formation, and calcifications can occur due to injection of large volumes into a single area or injecting fat into poorly vascularized areas resulting in failure of "graft take" with palpable mass formation resulting from fat necrosis which may be difficult to distinguish clinically from local recurrence in breast cancer patients and lead to a need for additional imaging and needle biopsy (3–15%). Moreover, post-lipofilling calcification can be found in mammogram (0.7–4.9%).
- Infection (0.6–1.1%).
- Under-correction or overcorrection of deformity.
- Damage to underlying structures, e.g., breast implants and pneumothorax.
- Intravascular injection with fat embolism.

39.3.2 Donor Site Complication

Complications appear to be minimal and related to liposuction technique. The possible complications include bruising, swelling, hematoma formation, paresthesia or donor site pain, infection, hypertrophic scarring, contour irregularities, and damage to underlying structure such as intraperitoneal or intramuscular penetration of the cannula.

39.4 Oncological Safety [4, 24–35]

Experimental studies have shown that adipocytes can stimulate breast cancer cells. Adipokines are factors that can stimulate breast cancerous cells through endocrine, paracrine, and autocrine pathways; theoretically, the "tumor stroma

interaction" can potentially induce cancer reappearance by fueling dormant tumor cancer cells in the tumor bed. There is increasing evidence that obesity, an excess accumulation of adipose tissue occurring in mammalians when caloric intake exceeds energy expenditure, is associated with an increased frequency and morbidity of several types of neoplastic diseases, including postmenopausal disruption of the energy homeostasis results in obesity, inflammation, and alterations of adipokine signaling that may foster initiation and progression of cancer. Other recent studies, some of which are based on endogenous WAT expressing a transgenic reporter, showed a significant level of adipose cell contribution to tumor composition. However, WAT contains several distinct populations of progenitors, and these data were obtained using crude or mixed cell populations. We therefore decided to purify by sorting the two quantitatively most relevant populations of WAT progenitors (endothelial cells and adipose stromal cells, ASC) and to investigate in vitro and in vivo their role in several orthotopic models of local and metastatic breast cancer. Compared with bone marrow-derived CD34+ cells mobilized in blood by granulocyte colony-stimulating factor (G-CSF), purified human WAT-derived CD34+ cells were found to express similar levels of stemness-related genes and significantly increased levels of angiogenesis-related genes and of FAP-α, a crucial suppressor of antitumor immunity. In vitro, WAT-CD34+ cells generated mature endothelial cells and endothelial tubes. In vivo, the co-injection of human WAT-CD34+ cells contributed to tumor vascularization orthotopic and significantly increased tumor growth and metastases in models of human breast cancer in nonobese diabetic severe combined immunodeficient (NOD/SCID) interleukin-2 receptor γ (IL-2Rγ)-null (NSG) mice.

The oncological safety should be considered as a priority problem for lipofilling. Many studies are showing the safety of lipofilling such as the multicentric study (Milan-Paris-Lyon) performed by Petit et al. dealing with 646 lipofilling procedures performed on 513 patients. The average interval between oncologic surgical interventions and lipofilling was 39.7 months. Average follow-up after lipofilling was 19.2 months. They observed a low complication rate; the overall oncologic event rate was 5.6 percent (3.6 percent per year). The overall locoregional event rate was 2.4 percent. Petit et al. reported a retrospective matched cohort study on 321 consecutive patients operated for primary breast cancer who subsequently underwent lipofilling for reconstructive purpose. The median follow-up of 56 months from primary surgery and 26 months from the lipofilling had shown no significant local recurrence when compared to 642 patients as a control group. However, there is a trend of higher risk of local event in subgroup of ductal carcinoma in situ. In 2010, Rietjens et al. reported a series of lipofilling procedures in breast cancer treatment and reconstruction. They followed 158 patients and found that postoperative complication rates are very low (3.6 percent) and that there is little alteration in post-lipofilling mammographic finding (5.9%). Seth et al. made a retrospective comparative study on 886 patients (1202 breasts) from 1998 to 2008 and revealed no significant differences in demographics, operative characteristics, tumor staging, or radiation therapy exposure between fat grafting (n = 90 breasts) and nonfat grafting (n = 1112 breasts) patients. Ninety-nine fat-grafting procedures were performed an average of 18.3 months after reconstruction, with only one complication (fat necrosis); they concluded that fat grafting did not affect local tumor recurrence or survival when compared with nonfat-grafted breasts. In 2007, the French Society of Plastic Surgery (SOFCPRE) announced that that they would not support the use of lipofilling for treating defects resulting from breast-conserving treatment (BCT) as a result of the lack of evidence on its oncological safety. A phase III multicenter randomized, controlled trial is currently taking place in France with the goal of investigating this issue. Also the American Society of Plastic Surgeons had set up a task force in 2009 (ASPS Fat Graft Task Force) to assess the indications, the safety, and the efficacy of autologous fat grafting on 283 patients; it showed the risk of malignancy with lipofilling could not be identified due to lack of standardized techniques and randomized controlled trials. Even though lipofilling seems to be a safe procedure in breast cancer patients, longer follow-up and further experiences from large multicentric oncological series are urgently required to confirm these findings [38–41].

Conclusions

Lipofilling in breast cancer surgery can be performed as a day surgery procedure, and it has acceptable efficacy in correction of deformities without compromising oncological outcomes. Although an apparent increase of local recurrences was observed at the IEO in the intraepithelial breast cancer patients, two recent match-control studies showed the cancer safety of lipofilling [36, 37]. More studies are needed to confirm that the risk of stimulation of local recurrences observed in the experimental setting is not observed in breast cancer patients. Moreover, application of experimental and fundamental researches on tissue engineering and stem cells can carry more hopes to augment the role of lipofilling in the future.

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Breast Reconstruction with Biological and Non-biological Meshes and Matrices

40

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40.1 Introduction into Meshes and Matrices in Breast Reconstruction

The terms mesh and matrix when applied to breast reconstruction are generally in reference to the composition of the material used in the manufacture of the product. The general consensus is that the term "matrix or matrices" refers to a product derived from biological sources (e.g. dermis), whereas "mesh or meshes" refers to a product wholly manufactured from synthetic materials (e.g. polypropylene). With the exception of SERI™, made from silk-derived bioprotein, the majority of products used in breast reconstruction can be divided into matrices and meshes.

The use of matrices in prosthetic breast reconstruction began with the publication of two papers in 2005 and 2006 by Breuing and Salzberg, respectively [1, 2]. Both authors reported small case series (n = 76; n = 20 breasts) using the human dermal-derived matrix Alloderm, in immediate single-stage implant breast reconstruction. Authors described a novel technique which aimed to shorten the reconstructive process by reducing prosthetic reconstruction from two-stage to one-stage immediate reconstruction. Since then, the use of meshes and matrices in breast reconstruction has gained in popularity and has provided the surgeon with the option for immediate single-stage implant reconstruction with mesh placement as a viable reconstructive option [3]. Single-stage techniques benefit patients by avoiding the need for repeated outpatient appointments for tissue expander fills and a second-stage operation. Meshes and matrices have been successfully employed both in single-stage implant-based breast reconstruction and in the setting of two-stage expander-based prosthetic reconstruction, nipple-sparing mastectomy (NSM) and skin-sparing mastectomy (SSM) [4–8].

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40.2 Mechanics of Meshes and Matrices in Breast Reconstruction

Meshes and matrices in prosthetic breast reconstruction are placed at the inferior border of the reconstruction. Prosthetic devices (implant or expander) are placed submuscularly (pectoralis major ± proximal rectus abdominis, serratus anterior). When partial muscle coverage is performed, pectoralis major is released off the chest wall. The subpectoral plane is developed to the second rib superiorly and to the sternal muscle fibres medially. The pectoralis major is then released and the mesh/matrix can be attached. Meshes/matrices are sutured medially from the inferomedial border of the pectoralis major to the medial border of the inframammary fold (IMF). The mesh/matrix is then sutured from the inferior border of the muscle to the chest wall fascia at the level of the IMF to increase the inferior aspect of the subpectoral pocket; most commonly interrupted sutures are used. A hammock of mesh is created for the prosthesis (Fig. 40.1).

The mesh/matrix is sutured laterally to ensure minimal lateral migration of the device. Some surgeons will perforate the matrix (meshes are already porous) with the aim to minimise fluid pooling around the device [9]. Many surgeons will facilitate fluid evacuation by placing a vacuumed drain between the skin flap and the mesh/matrix to reduce the potential deadspace between the two layers. Drains are left in situ until there is minimal drainage (<30 ml/24 h) to prevent seroma formation. Soft tissue compression dressings can also be employed to help reduce seroma formation in the immediate post-operative period:

1. *Meshes act as an inter-positional graft between the pectoralis major and IMF.*

   In all cases, the mesh allows the surgeon to release the pectoralis major and use the mesh as an extension of this inner tissue plane between the pectoralis lamellae and the outer skin envelope. This is especially useful in patients where the muscle sits high on the chest wall or is too tight which would limit the reconstructive volume [10].

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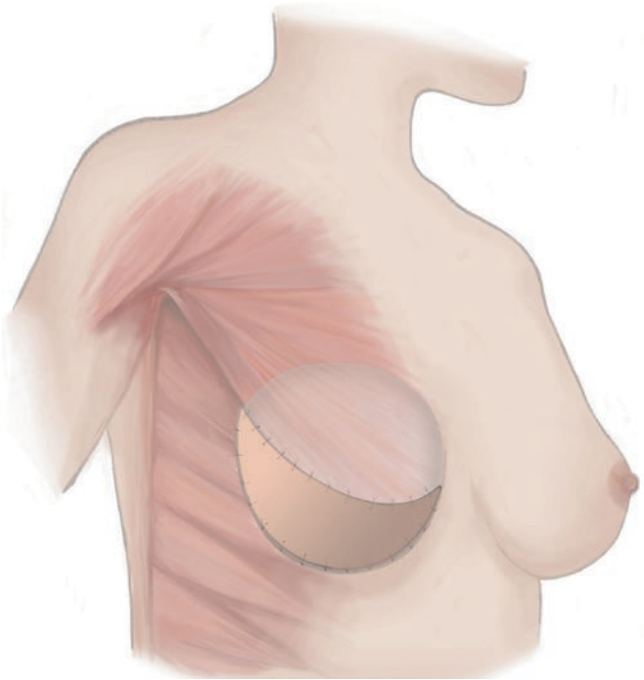

Fig. 40.1 Picture of mesh in breast reconstruction: schematic

The use of meshes enables a greater implant or expander volume to be placed at the original operation to either shorten the second-stage expansion process due to its ability to expand the subpectoral pocket or to remove the second stage entirely [11–13]. In obese patients with large ptotic breasts, there is often a discrepancy between the skin envelope of the patient and the underlying subpectoral pocket. Meshes be can be employed to redress this imbalance and improve the projection vector of the breast [10, 14].

2. *Meshes help to define and control the reconstructive pocket*.

   The use of a mesh along the IMF enables the surgeon to define the inferior aspect of the reconstructive pocket. This control can prevent implant migration and improve aesthetic outcomes by clearly defining the IMF [15–18]. The mesh also acts as a hammock to support the implant/expander and therefore maintain lower pole projection and a natural breast mound shape. Small case series have reported improvements in breast contour, implant placement, lower pole projection and IMF definition using blinded-surgeon assessors [18].

3. *Biological meshes may improve host neovascularisation of the mastectomy skin envelope*.

   Biological meshes may act as a stimulus to surrounding tissue including the skin flap, enhancing neovascularisation via the induction of growth factors in the surrounding host tissue (basic fibroblast growth factor [bFGF], vascular endothelial growth factor [VEGF] and transforming growth factor-beta 1 [TGF-β1]) [19]. Microcirculatory analysis in animal models demonstrated angiogenesis at 4 weeks post implantation on the skin-flap surface with established vasculature on the mesh observed at 8 weeks [20].

4. *Meshes confer additional soft tissue coverage to the prosthesis*.

   The position of the mesh at the infero-lateral pole enables the mesh to provide soft tissue coverage of the prosthesis. This reduces the need for extensive musculofascial dissection. Studies have reported that the increased soft tissue coverage supplied by the mesh can result in reduced capsular contracture around the implant with adjuvant radiotherapy [21, 22]. In addition, by increasing the tissue coverage of the prosthesis, the mesh can act to reduce contour irregularities (rippling, palpable implant) in patients with little subcutaneous fat in the mastectomy flap, thus improving aesthetic outcome [18].

40.3 Types of Meshes for Breast Reconstruction

Various companies have developed a number of meshes for use in breast reconstruction. Types of meshes in breast surgery can be divided into non-biological (synthetic) and biological types (Table 40.1). The majority of surgeons employ the use of biological meshes (also known as acellular dermal matrices/ADMs) in breast reconstruction as they provide a scaffold for host tissue ingrowth [23]. Training provided by manufacturers on the correct use of their product to surgeons and their continued support in its use can influence surgeons' choice of mesh, particularly when there is a learning curve associated with mesh use in breast reconstruction [24, 25].

40.3.1 Non-biological

Non-biological (synthetic) meshes have been used in breast surgery for over 20 years. They are manufactured using a variety of polyglactin, polyglycolic and polypropylene composites. By varying the composition of the mesh, manufacturers can alter its mechanical tensile properties and ability for the body to absorb the mesh. Non-biological meshes can be permanent or absorbable. Although the recent trend in reconstructive breast surgery has been in favour of biologic meshes, non-biological meshes still hold a role in prosthetic breast reconstruction.

Non-biological meshes can be divided into absorbable and non-absorbable meshes. Absorbable meshes in breast surgery include Vicryl (polyglactin 910), Dexon (polyglactin), GalaFLEX (poly-4-hydroxyalkanoate) and TIGR Matrix (copolymer of glycolide- and lactide-degrading fibres

Table 40.1 Soft tissue support meshes and matrices available for breast surgery

Type of soft tissue support	Product details	Clinical evidence
Synthetic		Case series
Absorbable		
Vicryl	Polyglactin 910	*n* = 24; 76 (Loustau et al. 2007; Tessler et al. 2014)
Dexon	Polyglactin	
GalaFLEX	Poly-4-hydroxyalkanoate	
		n = 3 (Adams 2014)
TIGR matrix	Copolymer of fast (glycolide) and slow (lactide) degrading fibres with trimethylene carbonate	*n* = 62 (Becker et al. 2013)
Non-absorbable		
Seragyn BR	Bicomponent polyglycolic acid (absorbable) caprolactone/polypropylene (non-absorbable)	*n* = 23 (Paepke et al. 2012)
TiLoop Bra	Titanized polypropylene	*n* = 231 (Dieterich et al. 2013)
Prolene		
PTFE	Polypropylene	*n* = 6 (Amanti et al. 2002)
Mersilene	Polytetrafluoroethylene	*n* = 1 (Coelho et al. 2012)
	Polyester	*n* = 67 (Reitjens et al. 2005)
Biological		
Animal derived		
Strattice	Porcine dermis	*n* = 104 (Salzberg et al. 2013); *n* = 200 (Lardi et al. 2014)
Surgisis	Porcine small intestine mucosa	Centeno 2009
Surgimend PRS	Bovine foetal and neonatal dermis	*n* = 222 (Butterfield 2013)
Veritas	Bovine pericardium	
Cadaveric derived		*n* = 93 (Mofid et al. 2012)
Allomax (Neoform)	Human dermis	*n* = 31 (Losken 2009)
DermaMatrix	Human dermis	*n* = 62 (Brooke et al. 2012)
Alloderm	Human dermis	*n* = 105 (Weichman et al. 2013)
FlexHD	Human dermis	*n* = 97 (Liu et al. 2014)
Silk derived		
Absorbable		
SERI	Silk-derived bioprotein	See results, Table 3
Autologous		
Dermal graft	Abdominal harvest site via mini-abdominoplasty	*n* = 21; 76 (Hudson et al. 2012; Lynch et al. 2013)

with trimethylene carbonate). Non-absorbable meshes include Seragyn BR (bicomponent polyglycolic acid (absorbable) caprolactone/polypropylene (non-absorbable)), TiLoop Bra (titanium-coated polypropylene mesh), PTFE (polypropylene) and Mersilene (polytetrafluoroethylene polyester) [26]. Recently, studies have revisited the use of non-biological meshes in breast reconstruction as they have an established safety profile and cost considerably less than biological meshes [27, 28]. There are no published comparative studies with synthetic versus biological meshes at present. A hybrid biological synthetic mesh, SERI surgical scaffold (Allergan Inc., MA) was recently introduced as a cross-over mesh between synthetic and biological. It is a scaffold made of silk bioprotein, fibroin (Biosilk), which provides strength mechanics similar to synthetic meshes whilst remaining absorbable [29].

40.3.2 Biological

Biological meshes are tissue-derived manufactured meshes. Tissue is processed from a variety of human cadaveric and animal sources to remove the epidermis and cells resulting in an "acellular" matrix (Table 40.1). This is also referred to as ADM (acellular dermal matrix). The majority of biological meshes used in breast reconstruction are derived from processed dermis (cadaveric, porcine, bovine). Other tissue sources include porcine small intestine mucosa (Surgisis) and bovine pericardium (Veritas). Proprietary processing, using a variety of techniques unique to each product, removes the epidermis (in the case of dermal-derived tissue) to leave a non-cross-linked acellular dermal matrix (e.g. Alloderm (LifeCell Corporation, NJ), FlexHD (Ethicon Inc., Somerville NJ), DermACELL (LifeNet Health, VA), DermaMatrix (Synthes, PA), Strattice (LifeCell

Corporation, NJ)) [23]. The resulting mesh is reported to have minimal antigenic properties; however, patients should be counselled in their consent process regarding the mesh composite and carefully screened for previous allergies. A "red breast syndrome" has been reported by Ganske et al. [30]. Patients presented with skin erythema overlying the dermal matrix with punch biopsies of the tissue revealing a delayed-type hypersensitivity reaction suggesting a localised immune host response requiring corticosteroid treatment [30].

Biological meshes have been widely used in immediate and two-stage breast reconstruction in recent years. No head-to-head randomised controlled trials have been completed to date to establish complication rates between the different meshes. Small case series comparing different meshes have been published although these are frequently underpowered and subject to reporting bias; therefore, no clear conclusions can be drawn between the different products on the market. A disadvantage to using biological meshes is that at current market pricings, the direct cost of biological meshes does not appear to offset economic savings related to greater expander fill volumes and a reduction in revision surgery [31, 32]. However, some argue that when the cost-effective incremental cost utility of biological meshes is calculated (due to an increase in quality-adjusted life years with mesh reconstruction), biological meshes can be considered cost-effective [33].

40.4 Why Choose Biological Matrices Over Synthetic Non-biological Meshes?

A recent survey of plastic surgeons within the USA demonstrated that the majority of respondents (84.2%, n = 361) used ADM in breast reconstruction in preference to synthetic meshes [34]. The reasons for this are multifactorial.

There is a paucity of clinical data on the use of synthetic meshes in breast reconstruction in the literature. The majority of data exists for the following meshes: TiLoop and TIGR meshes. The largest case series for TiLoop mesh was reported by Dieterich et al. [35]. Two hundred thirty-one breasts were operated on in primary and delayed implant-based reconstruction. The overall complication rate was 29%. Similarly the TIGR mesh case series by Becker and Lind [36] reported on 112 breasts with a complication rate of 19% in primary breast reconstruction. TiLoop Bra has been associated with a host granulomatous reaction in the skin flap (estimated at 4%) which in the context of recurrent DCIS can create an oncological challenge for cancer surveillance [37]. In addition, in patients with thin skin flaps, there have been reports of TiLoop mesh rippling and palpability [38]. The lack of clinical data on synthetic meshes limits the surgeons' ability to evaluate the product, and therefore it is less in use.

By contrast there are a much greater number of papers reporting on biological matrices in breast reconstruction with longer-term follow-up and larger numbers (n = 6199 cases) [58]. Pharmaceutical industry sponsored research on new biological meshes which influences the surgeons' choice of product. Marketing strategies and keynote speeches from industry-funded surgeons may influence the surgeons' choice of product although it is difficult to quantify. The overriding influence on product choice remains based on the reported lower complication rates with matrices compared with meshes.

Current estimates from meta-analysis of reconstructions with ADM give a pooled complication rate of 18%; however, more recent case series have reported lower rates in experienced surgeons of 5.3–8% [39, 40]. Moreover, the risks of total complications in a recent meta-analysis on ADM use in breast reconstruction concluded that the risks of implant loss and total complications were not significantly different from submuscular implant reconstruction without ADM [40].

40.5 Complications associated with Meshes in Breast Reconstruction

Amongst published literature there is conflict surrounding the overall complication rates associated with meshes in primary breast reconstruction. A number of meta-analyses have been performed by various groups in order to establish if the presence of mesh in the reconstruction confers an increased complication rate to the patient [12, 21, 41–44]. Unfortunately, there is a lack of level I evidence to evaluate this topic, with the majority of studies being small numbered comparative or case series [44] and limited by heterogeneity in study design, outcome measurement, selection and reporting bias [44]. As such, any pooled data from non-randomised studies for meta-analyses on complication rates with meshes has limited validity. In addition, a number of the studies published are funded by companies manufacturing the meshes which leads to considerable publication bias [44]. Only one randomised controlled study has been conducted to date which was stopped early due to poor recruitment [45]. The median complication rate following ADM-assisted immediate breast reconstruction was calculated by Potter et al. as 18% (6–64%), compared with 14% (5–45%) in a standard two-stage expander reconstruction without mesh [44]. There is a general consensus between the systematic reviews on this topic that the use of meshes in breast reconstruction does confer increased overall complication rates; however, the magnitude of this effect remains ill-defined. Comprehensive prospective randomised controlled trials are needed to investigate the true effect of meshes on complication rates. It has

been suggested that the differing complication rates may reflect not only a surgical learning curve for the technique [24, 25] but also variations in patient selection [11].

40.6 Complications Within the Post-operative Period Following Breast Reconstruction Using Matrices and Meshes and How to Manage Them Clinically

1. *Infection*

Despite the aseptic techniques used intraoperatively to prevent infection of the mesh (irrigation in antibiotic solution, changing of surgical gloves intraoperatively, minimal mesh and tissue handling, post-operative intravenous antibiotics) as a foreign body, the mesh can act as a nidus for bacterial ingrowth. Observational data suggests there is an association with infection rate and high intraoperative expander/implant volumes greater than 50%of the total volume; however, this needs to be corroborated [46].

Infection will present as classical signs of inflammation at the site of surgery with purulent discharge, wound swab or blood-positive cultures for bacterial infection and elevated white blood cells and C-reactive protein. This is in contrast to the observed and reported "red breast syndrome" [30] which may present with signs of inflammation without pain and infection markers and cultures will be negative. This condition will settle without antibacterial treatment and can be managed with close observation and simple anti-inflammatory agents.

If diagnosed early, infection may be treated conservatively with intravenous or oral antibiotics; however, if established the patient will require removal of the infected mesh as a secondary operation with removal of the prosthesis. Common organisms associated with implant infections include *S. epidermidis*, *S. aureus*, *S. marcescens* and *P. aeruginosa* [47]. There is no consensus in the use of prophylactic intraoperative and post-operative antibiotics; however, a 5-day course of oral antibiotics post-operatively is a common regimen. Removal of the matrix is surgeon dependent; however, it can be based on its intraoperative appearance. If the matrix appears partially hydrolysed, non-viable or inflamed, then the authors recommend its removal. A recent systematic review found infection rates with mesh reconstructions to vary from 0% to 31% with a combined average of 11.6%. Of note, in skin-sparing mastectomy the natural barrier to infection is compromised, and infection in the post-operative period must be considered [48]. There is a shift in practice to the use of sterile ADM (versus aseptic ADM), which involves terminal sterilisation in an attempt to reduce infection rates further [49].

2. *Haematoma and Seroma*

Although biological matrices are processed to prevent a host antigenic reaction to their implantation in the tissue, they do however cause a tissue reaction which predisposes this type of breast reconstruction to increase seroma formation. In addition, the increased reconstructive pocket volume created by the mesh at the lower pole can create an increased deadspace in the reconstruction which may predispose patients to haematoma and seroma formation. Interestingly, there is a correlation between increasing surface area of the matrix used and the odds of seroma formation [46]. A minimised surgical technique has been described (patching only the lateral area of the reconstruction with a small surface area of ADM; $n = 225$) which reports a reduction in seroma from 3% to 0%, although these results need further validation [50]. Extensive use of electrocautery has also been reported as a risk factor for seroma formation [51].

Judicious use of vacuumed drains post-operatively, soft compression dressings and surgical bras have been effective to reduce the incidence of seroma in these patients, although the drain site must be kept as clean as possible and ideally tunnelled in a long subcutaneous tunnel to minimise exposure of the implant and matrix to outside bacteria [52].

One study reported with these measures that a reduction of 18.6–4.7% ($p = 0.0022$) in seroma was achieved [53]. Seroma formation may also be due to insufficient intraoperative expansion when using a tissue expander as this will also allow fluid to collect in the reconstructive pocket. However, increased intraoperative fill volumes must be carefully considered as the risk of explantation increases with volumes over 300 cc due to increased skin-flap tension which can compromise vascularity especially if the patient has additional risk factors for complications [46].

Drains are often placed to prevent fluid accumulation and removed once drainage is less than 20–30 ml in 24 h. Low-grade seromas can be managed with percutaneous ultrasound-guided drainage; larger seromas and haematomas may require surgical evacuation.

3. *Mastectomy Flap Necrosis and Reconstructive Failure (Explantation)*

Skin-flap necrosis with mesh-assisted prosthetic breast reconstruction has been attributed to mastectomy flap thickness and disruption of the subdermal plexus during surgery. Prior to ADM use, mastectomy flap necrosis was as high as 15%. The additional burden of the mesh on tissue oxygenation demands has been postulated as a reason for the increased flap necrosis rates. Meta-analyses have reported an approximate twofold increased risk in recon-

structive failure with mesh-assisted reconstructions [54, 55] although their conclusions are based on limited evidence.

Minor wound dehiscence and matrix exposure can be managed in the outpatient setting with dressings and close monitoring, but may necessitate wound closure in theatres. Larger areas of skin-flap necrosis will require a return to theatres for debridement plus/minus removal of the matrix and implant in some cases. Placement of an expander will be required to facilitate wound closure.

Increased explantation rates observed with ADM-assisted breast reconstructions may also be in part due to prosthesis removal if mesh removal is required with severe infection. This may not be fully represented in the data reported. Although the mesh or matrix provides additional cover to the implant should skin necrosis occur and in smaller areas of necrosis, the implant can be salvaged. An alternative to explantation and delayed reconstruction in cases of matrix infection has been described. In this technique the implant and matrix are removed, and a negative pressure sponge is placed into the cavity. This maintains the reconstructive pocket until the infection is cleared and the patient can have replacement of the implant on the same hospital admission [52].

4. *Management of the Aesthetic Profile*

Clinical follow-up in the post-operative period includes clinical assessment of the reconstruction at 2 weeks, 3 months, 6 months and 12 months in general. During this time the reconstructed breast will begin to soften and develop into its long-term shape. Repeated aesthetic assessment is required to monitor for the need of further procedures to optimise the end aesthetic result. Autologous fat grafting (lipofilling) of the ADM-assisted implant reconstruction is often required to the upper pole to aid with implant coverage and volume especially in patients with a low BMI. Symmetrisation procedures on the contralateral native breast are often performed in unilateral reconstructions.

40.7 Risk Factors for Complications associated with Meshes in Breast Reconstruction

Since the advent of meshes in prosthetic breast reconstruction, a number of relative contraindications for the technique have been developed with increasing surgical experience [56].

Patients with a high body mass index (BMI) of over 30 and long ptotic breasts are at increased risk of poor wound healing as long skin flaps are prone to ischaemia and infection [11, 24, 46, 57, 58]. In addition, the thickness of the subcutaneous layer (>3 cm in some cases) can prevent significant projection from the prosthesis, impairing aesthetic results and adherence of the mesh to the subcutaneous layer [7]. Patients who have undergone pre-reconstruction radiotherapy to the chest wall are at increased risk of impaired skin-flap perfusion and post-radiotherapy fibrotic changes [7, 46]. The addition of mesh within a prosthetic reconstruction in these patients should be assessed on an individual basis intraoperatively based on skin-flap perfusion to prevent skin-flap necrosis (clinical assessment ± laser Doppler or laser-assisted indocyanine green imaging) [59]. Similarly, heavy smokers are at higher risk of necrosis due to impaired microvasculature and wound healing and therefore reconstructive failure with or without mesh [58]. This increased risk for reconstructive failure has been estimated at up to five times that of non-smokers in prosthetic reconstruction [60]. In addition, patients with non-nipple-sparing mastectomy, implant volumes of 600 ml or greater and age over 50 years are also at increased risk of post-operative complications ($p < 0.001$) [39]. Positive sentinel lymph node sampling and post-operative radiotherapy are not prohibitive to mesh use. Careful assessment of skin-flap perfusion intraoperatively is key to help minimise post-operative complications.

In all patients being considered for mesh-/matrix-assisted prosthetic breast reconstruction, an assessment of skin-flap perfusion should be made intraoperatively. If there is any concern regarding perfusion, the use of biological or synthetic meshes would be contraindicated. In patients with multiple risk factors for complications, an alternative reconstructive option must be considered.

40.8 Patient Selection for Mesh-/Matrix-Assisted Breast Reconstruction: Achieving Good Results

Patient selection is paramount in ensuring a good reconstructive result from ADM-assisted implant reconstruction. The technique is not without its pitfalls, and a considered approach should be used when deciding if the patient is suitable for this technique. The procedure is only 10 years from its conception, and as such, we have limited long-term data for this operation. Similarly, biological matrices in particular have unpredictable long-term characteristics, and as such, in young patients where the reconstruction will require longevity and consistency, autologous reconstructive options must be considered first. Although, as stated previously, the complication rates for ADM-assisted implant reconstructions have reduced with time and surgical experience, they are still considerable with a total rate ranging from 6 to 64%. With the experienced surgeon who is used to this form of reconstruction, the complication rate will be low; however, there is a considerable learning curve to this procedure [24]. This may explain the unacceptably high complication rates reported. Complications may delay adjuvant therapy and cause the patient considerable

psychological stress with repeated procedures especially if faced with reconstructive failure. Although the benefits and good outcomes with this technique are well documented and surgical practice has shifted to ADM-assisted implant reconstruction, it must be remembered that there is little level I evidence to support this technique.

A number of papers have been published presenting surgeons with treatment algorithms to aid the decision in placing mesh when performing prosthetic breast reconstructions [11, 25, 61]. Good aesthetic results with mesh-assisted tissue expander/implant reconstruction have been achieved in the following patients:

- Low BMI (<30)
- Non-smokers
- Small pre-operative cup size
- Patients <50 years old
- Patient unsuitable or refusing autologous reconstruction

In summary, careful patient selection is the most important step prior to ADM-assisted breast reconstruction and will determine in part, along with surgical technique and skill, the overall reconstructive outcome.

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How to Manage Complications in Breast Reconstruction

41

Francesca De Lorenzi

Breast surgery is associated with a very low postoperative complication rate and even lower mortality rate. The reported morbidity rates range between 2% and 49% and are increased in cases of axillary surgery and immediate breast reconstruction [1, 2]. In case of reconstruction, the complication rate is still low and does not result in higher readmission rates compared with mastectomy only, therefore supporting the broadened access to reconstruction [3]. Furthermore, immediate reconstruction has been shown to be safe in terms of surveillance for recurrent cancer [3] and timing of systemic treatment delivery [4].

Even though complication rate is low, it represents an important indicator of the quality of surgical care, and, in an era of heightened patient awareness, healthcare providers are striving to identify and implement methods to reduce postoperative complications and ensure patient safety. An accurate preoperative planning is crucial to reduce surgeon's mistakes that can lead to complications. Preoperative evaluation includes drawings, measurements and pictures with the patient in the standing position and the assessment of comorbidities, smoking status and other potential risk factors for complications. These criteria lead surgeons to a tailored reconstructive option for every patient.

When complications occur, their quick identification and an appropriate management are mandatory to lower patient morbidity and improve final outcomes. Not all complications lead to surgical failure if adequately treated. Finally, a strict follow-up of patients with surgical complications is recommended, including an effective communication to patients and a good relationship, necessary to avoid legal litigations.

In this chapter we focus on the main complications after reconstruction and their management. Unsatisfactory results due to surgical mistakes or changing local situations, as volume and shape asymmetries, symmastia, dislocations of the inframammary fold and double-bubble deformities are not investigated.

41.1 Bleeding

It is the most common cause of reoperation in the early postoperative period, both in case of mastectomy and reconstruction or oncoplastic procedures, varying from 0.4% to 1.9% of the patients and generally within the first 4 days from the initial procedure [1].

Although in the vast majority of cases early postoperative bleeding requires surgical revision and haematoma drainage, in few cases a conservative approach may be sufficient, including compressive bandage and single intravenous administration of antifibrinolytic drug (tranexamic acid is the most used medication to prevent fibrinolysis). When fibrinolysis exceeds coagulation, unwanted surgical bleeding may occur despite adequate haemostasis. Because of uncertainty about the effect of tranexamic acid, particularly on vascular occlusive events, it is not recommended for routine use during most surgical procedures [5]. On the contrary, topical application of dilute tranexamic acid at the site of the wound reduces bleeding (Figs. 41.1, 41.2, 41.3) [6].

In case of implant reconstruction, haematoma formation enhances the risk of capsular contracture; therefore, surgical revision may be justified even in case of non-massive bleeding.

Patients with a bleeding disorder are at higher risk of return to operative room for haemorrhagic event, even though the vast majority of them can undergo surgery safely without complications. Balancing the timing of stopping or resuming anticoagulants, risk of embolic or thrombotic events and surgery can be challenging at times and often requires a multidisciplinary approach.

Late haematomas are also described both in case of implant reconstruction [7, 8] and flap donor site [9], presenting several months/years after the reconstructive procedure. Patients with implant present breast swelling and asymmetry generally associated to pain and no bleeding disorders. Many of these

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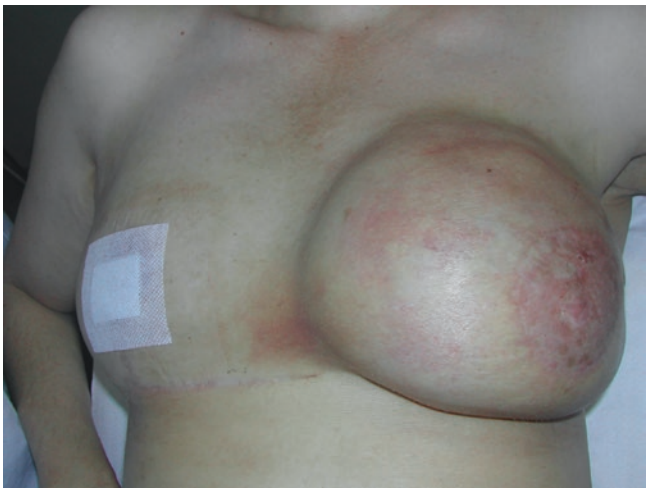

Fig. 41.1 Late haematoma of the left breast presenting 4 months after left reconstruction with implant. The patient described left breast swelling and progressive expansion. It required surgical revision

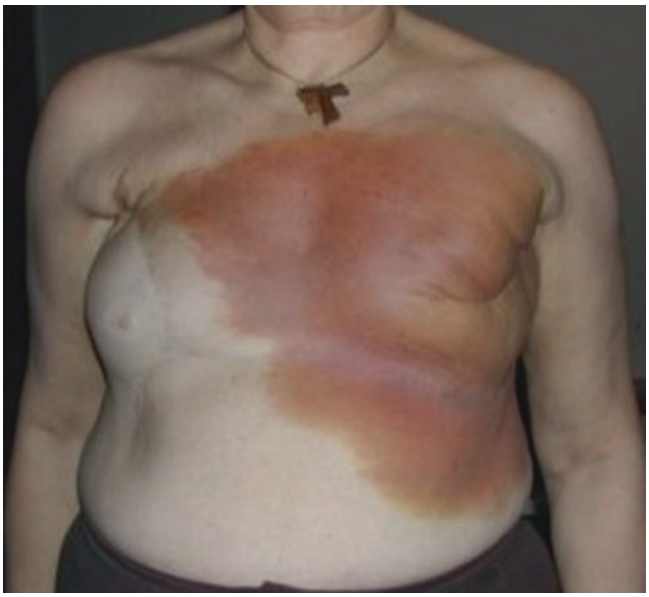

Fig. 41.2 Late haematoma of the left breast, no breast swelling is present, not requiring surgical revision

cases do not have a definitive mechanism of injury or develop symptoms immediately after the triggering event. Sudden enlargement onset or progressive expansions are both described. Ultrasound is an appropriate and cost-effective tool for differentiating between fluid collection and haematoma. Moreover, percutaneous ultrasound-guided needle drainage of fluid collection can confirm fluid nature, and the aspirate can be sent to pathology to rule out malignancy or infection. However, definitive treatment of late haematomas involves surgical drainage and capsulectomy and implant change (implant reconstructions) or sac excision (flap donor sites).

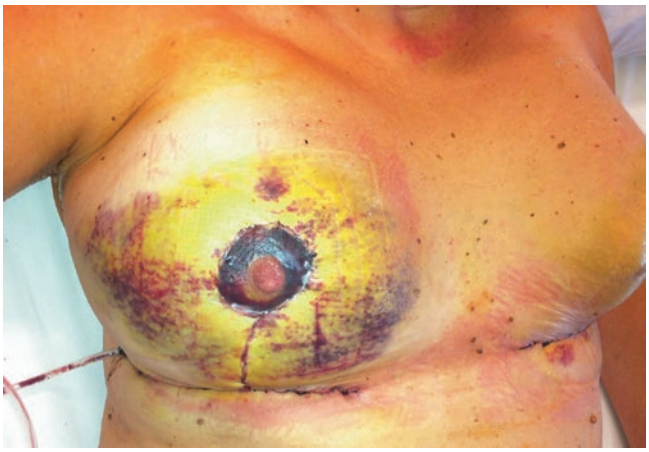

Fig. 41.3 Haematoma of the right breast after oncoplastic surgery conservatively managed

41.2 Surgical Site Infection

Surgical site infections at the donor or mastectomy site are the predominant cause of postoperative morbidity following mastectomy and immediate reconstruction [10]. Significant independent risk factors for infection were identified as BMI greater than or equal to 25 kg/m^2, chronic alcohol use, American Society of Anaesthesiologists classification of 3 to 5, flap failure in autologous or hybrid cases and operative time greater than 6 h [11]. The overall incidence rate of surgical site infection is 3.53% according to the US National Surgical Quality Improvement Program database (years 2005–2009). Patients within the dataset were divided in three groups according to the method of reconstruction: autologous procedures, prosthetic and hybrid type. Adjusting for confounding factors, there is no statistical difference in rates of surgical site infection among the three methods of reconstruction [12]. However, the gravity of surgical site infection is different among the three groups. In implant-based reconstructions, there is a risk of device loss and need for intravenous antibiotics. It generally requires reoperation with either removal of the implant or removal and replacement. Surgical site infection in autologous reconstructions typically does not result in loss of the flap but may result in deformity, the need for prolonged dressing changes and/or later reoperation (Fig. 41.4).

Focusing on infected implants, there is strong evidence that previous radiation therapy confers a significant risk of implant infection and suggestive evidence that simultaneous lymph node dissection increases the risk [13].

In the past, common practice was the immediate removal of the infected breast prostheses. However, the more recent literature has explored options for device salvage [14, 15, 16], changing surgical dogma that dictated foreign body removal in instances of infection. Methods for salvaging an

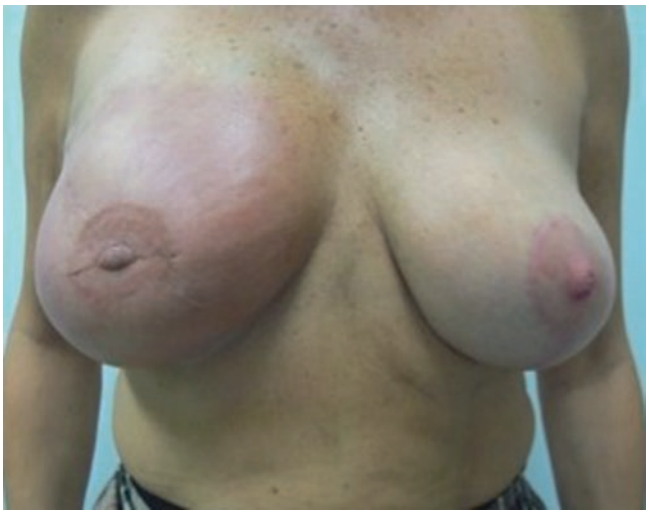

Fig. 41.4 Infection of the right breast after implant-based reconstruction

infected device have included systemic antibiotics combined with either conservative wound drainage or antibiotic lavage, capsulotomy/capsulectomy and device exchange, capsule curettage and continuous antibiotic irrigation. Treatment strategy is based on the response of the infection to initial antibiotic therapy and on the availability of soft tissue coverage (possible association to threatened or actual implant exposure). The severity of infection is an important factor in predicting the outcome of attempted salvage. The nature of infection may influence the ultimate outcome as well [17]. Organisms such as common skin flora may be treated with a high success rate, but organisms such as *Pseudomonas* species or Gram-negative rods may not be easily treated, and device removal is more likely indicated. Finally, if overwhelming localized infection or systemic sign of infection persists, further salvage attempts should be abandoned.

Finally, a further management strategy successfully indicated in case of mild infection and exposed implant is prosthesis explantation and immediate autologous reconstruction [18].

41.3 Necrosis and Wound Dehiscence

41.3.1 Managing Skin and Fat Necrosis After Oncoplastic Procedures

Glandular necrosis is the most challenging complication of oncoplastic procedures. Aggressive glandular undermining from both the skin and pectoralis muscle (dual-plane undermining) can lead to glandular necrosis in fatty breasts. Its incidence varies up to 13.4% [19, 20] in the literature. Imaging evaluation after oncoplastic surgery revealed fat necrosis in 18% of the cases on clinical examination, in 15% with ultrasound and 7% confirmed on pathology [21]. Non-healing wounds are recorded in 8.6% of the patients undergoing oncoplastic surgery [22]. These rates can be considerably reduced, incorporating the evaluation of breast density into the decision-making process [23]. In fact breast density predicts the fatty composition of the breast and determines the ability to perform extensive breast undermining and reshaping without complications. Breast density can be classified into four categories based on the Breast Imaging Reporting and Data System (BIRADS): fatty (1), scattered fibroglandular (2), heterogeneously dense (3) or extremely dense breast tissue (4) [24]. Low-density breast tissue with a major fatty composition (BIRADS 1/2) has a higher risk of fat necrosis after extensive undermining. Other risk factors for fat necrosis are diabetes, smoking habitus and previous irradiation of the breast.

Areas of fat necrosis can become infected and cause wound dehiscence resulting in postoperative treatment delay. They are usually managed conservatively, with daily dressing and antibiotic therapy. Surgical debridement is sometimes necessary to accelerate the healing process.

Fat necrosis can lead to scar retraction and deformities in the long term, therefore requiring surgical correction of sequelae.

41.3.2 Managing Flap Necrosis

It is due to an insufficient blood supply or drainage of the flap. Different mechanisms are responsible in case of pedicled or microvascular flaps, and the final result is the loss of part or the whole flap.

In case of pedicled transfer, flap necrosis is generally associated to peripheral venous congestion rather than arterial ischaemia. Important flap loss (greater than 25% of the transfer) is exceptional, and it may be related to preoperative risky factors as pre-existing scarring, smoking, obese and diabetic patients or thrombotic disorders. Massive abdominal flap necrosis can be also related to technical errors during the flap harvesting (traction of the pedicle) or insetting (twisting or torsion of the pedicle). Moderate flap loss (between 5% and 25%) occurs more frequently, and it is often related to venous congestion that will be the cause of necrosis. In both situations early surgery is recommended as soon as the limits of cutaneous venous congestion are well defined (generally on second postop day) and before thrombosis spreads to a larger portion of the flap. These situations generally require also late revisional surgery to correct the sequelae of the necrosis and eventual asymmetries with the contralateral breast (Fig. 41.5).

In case of minimal skin necrosis of pedicled flaps (less than 5% of the transfer), reoperations are not necessary, and the wound spontaneously evolves. Postoperative care is simple and generally managed by patients themselves.

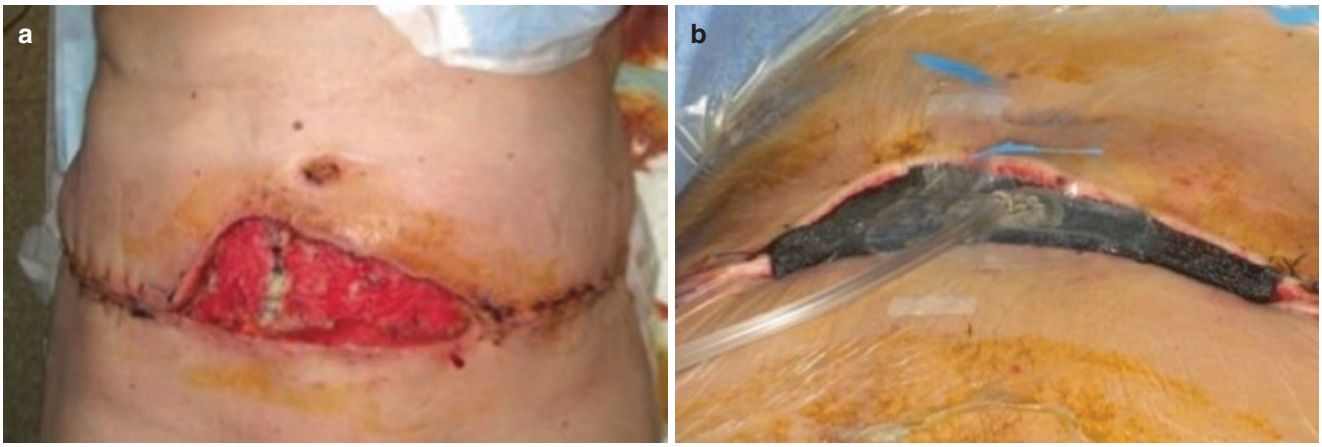

Fig. 41.5 Necrosis of the abdominal wall (**a**) after pedicled TRAM breast reconstruction. After debridement primary closure is not possible and negative-pressure wound therapy is a good solution (**b**)

In case of microvascular transfers, thrombotic complications are the major barrier to successful reconstruction, and they may be responsible of whole flap loss. For this reason, those patients candidate to microvascular transfers should be questioned about hypercoagulable history at the first consultation (family history of abnormal blood clotting, thrombosis in unusual sites, idiopathic or recurrent blood clots, a history of miscarriages, stroke at a young age). Unfortunately, thrombophilias remain a preoperatively silent and frequently undetected physiologic status against microvascular tissue transfer [30].

Routine postoperative care generally includes aspirin daily administration for coronary thrombosis risk reduction and potential enhancement of vascular patency as well as venous thromboembolism prophylaxis with subcutaneous heparin and sequential compression devices [31]. Microsurgical exploration is necessary for thrombotic events in about 7% of the cases [31]. It is due to arterial insufficiency (white and cold flaps, without turgor or Doppler signal) or venous insufficiency (blue and cold flaps, with fast capillary refill). Overall microvascular salvage rate is about 89% with a combination of intra-arterial, subcutaneous/intramuscular and intravenous tissue plasminogen activator, microvascular explorations and therapeutic multiagent anticoagulation/antiplatelet therapy.

41.3.3 Managing Mastectomy Flap Necrosis

It is a significant clinical morbidity after skin sparing and nipple and areola sparing mastectomies, related to the mastectomy itself and not to the reconstructive procedure. The incidence of native mastectomy skin necrosis after breast reconstruction ranges from 6% to 30% in retrospective series [25]. It is significantly higher in patients with higher mastectomy weight and body mass index, which correlates with breast size and subsequent mastectomy weight and in cases of diabetes. It is significantly more frequent in smokers, regardless of the type of reconstruction [26, 27]. Therefore, smokers undergoing reconstruction should be strongly urged to stop smoking at least 3 weeks before their surgery. Mastectomy incision type may influence its incidence [28].

Skin flap necrosis can be defined as mild (no intervention needed), moderate (requiring at least office debridement) or severe (surgical debridement needed, implant loss or healing not complete at 8 weeks) [29]. A not healed wound by 8 weeks postoperatively indicates a severe degree of ischaemia or wound-healing problems, which may be associated with infection and increased risk of dehiscence and can potentially delay adjuvant chemotherapy or radiotherapy (Figs. 41.6, 41.7, 41.8).

A different management is recommended in case of autologous or implant reconstructions. The benefit of autologous reconstruction is the possibility of healing by secondary intention with a well-vascularized flap at the base of the wound. This is in contrast to implant-based reconstruction where mastectomy skin loss increases the risk of implant exposure, necessitating earlier and more aggressive intervention with debridement and closure.

41.3.4 Managing Necrosis at the Donor Site

Necrosis of the anterior abdominal wall is observed typically in the infraumbilical region, which occasionally involves the umbilicus as well, and it is more frequent in smokers and obese patients [26, 32]. In fact, abdominal flaps are widely undermined and depend on a random blood supply unlike the axial vascular source of TRAM and DIEP flaps.

Similarly, in case of latissimus dorsi reconstruction, marginal necrosis of the dorsal wound may occur especially in case of closure with tension (extended latissimus dorsi flap).

According to different degrees and the extent of necrosis, wound care treatment modalities include debridement, wet-

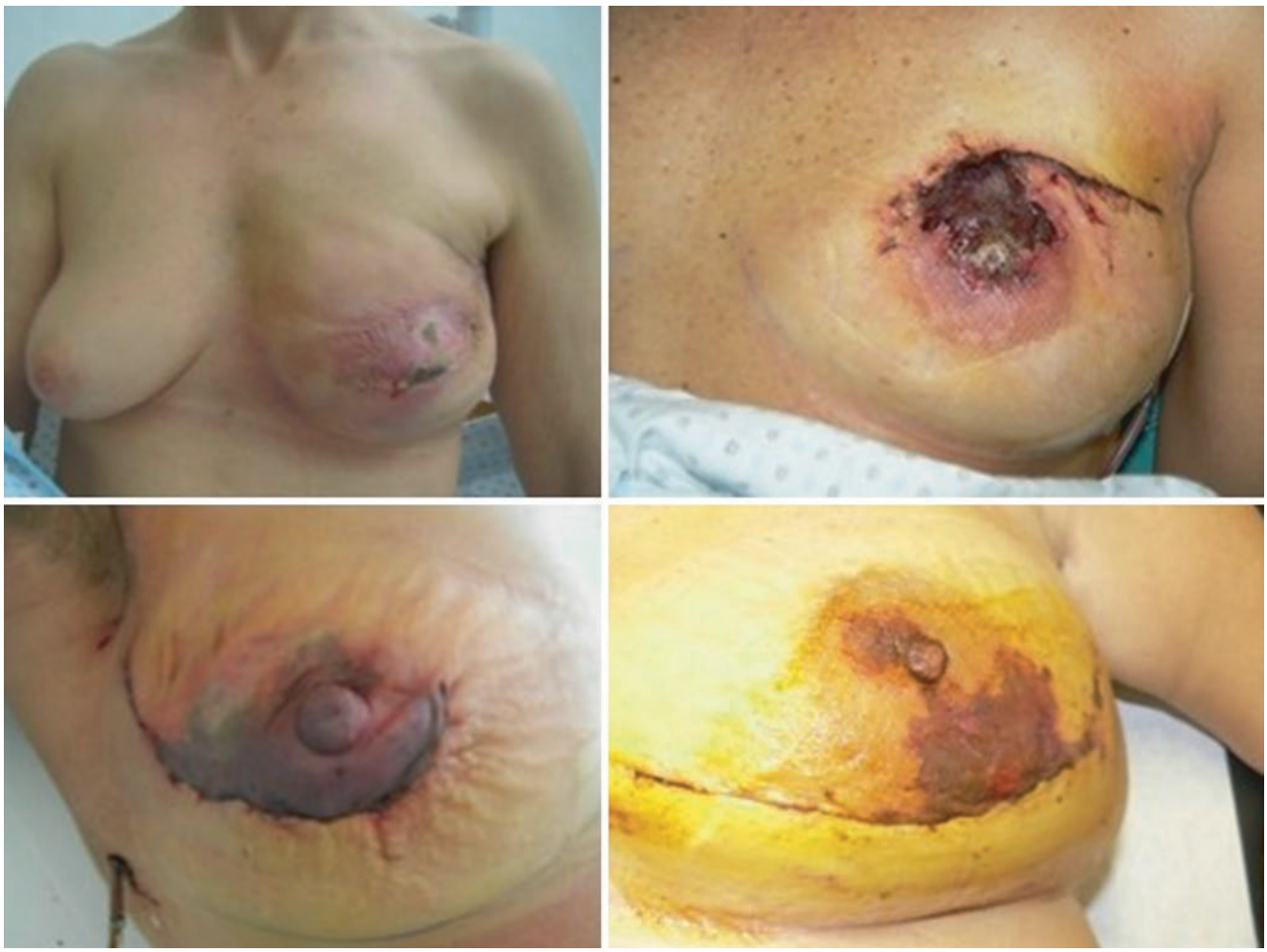

Fig. 41.6 Different degrees of mastectomy flap necrosis

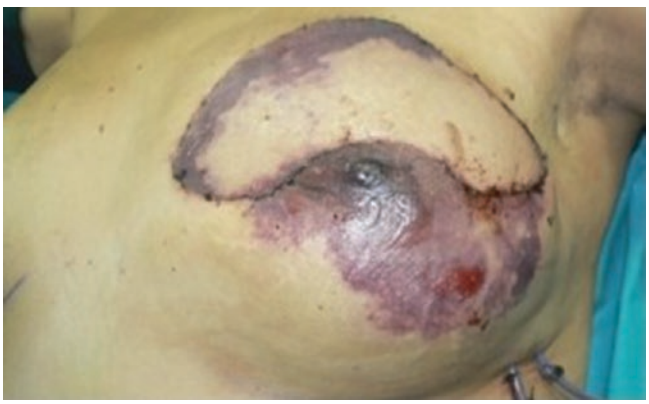

Fig. 41.7 Mastectomy skin flap necrosis and partial flap necrosis after left reconstruction with pedicled TRAM. Revisional surgery required

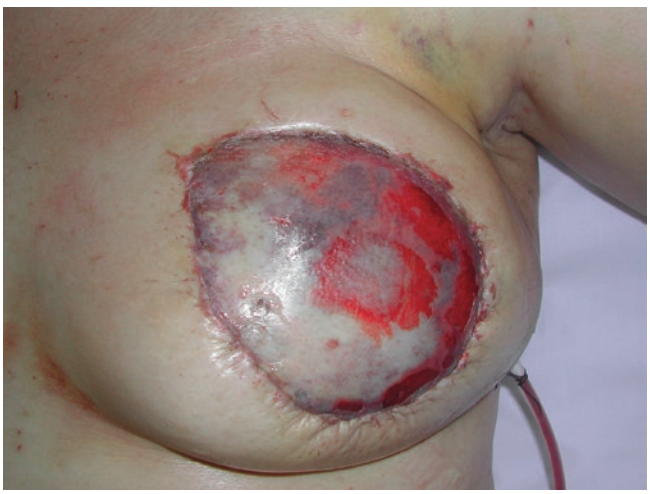

Fig. 41.8 Total flap necrosis after left reconstruction with microvascular transfer. Early microsurgical exploration failed

to-dry dressing changes and negative-pressure wound therapy. In case of massive necrosis, when primary closure is not possible after debridement, negative-pressure wound therapy may be an appropriate solution. The system generates negative pressure resulting in approximation of the wound edges, aspiration of infectious debris and exudates, reduction of oedema, increase in blood flow, promotion of granulation tissue generation and preservation of a wound-friendly moist environment [33].

In case of long-term chronic abdominal wound conservative treated, the risk of acute infection increases, and it may require the removal of the underlying abdominal mesh if present. If not, a chronic skin fistula may occur. If germs affect the mesh located behind the anterior rectus fascia, superficial wound debridement, even combined with adequate antibiotic therapy, is not adequate. The removal of the contaminated mesh is mandatory, which can weaken the abdominal wall.

In any case of healing problems at the donor site, revisional surgery may be necessary to correct scarring and deformities.

41.3.5 Pyoderma Gangrenosum

It is an inflammatory neutrophilic dermatosis characterized by painful, sterile ulcerations, bullae or pustules [34]. The aetiology is unclear, but it is thought to involve autoimmune dysfunction with dysregulation of the innate immune response. It is often associated with other autoimmune diseases as inflammatory bowel disease, rheumatoid arthritis, hematologic dyscrasias and connective tissue diseases. The so-called postoperative pyoderma gangrenosum is defined as the pathergic development of pathognomonic lesions in the surgical incision [35]. The typical presentation is an initial erythema of the recent surgical site, and afterwards the wound may dehisce or develop small punctate ulcerations that eventually coalesce, often associated with fever and leukocytosis. A wound infection is often diagnosed and antibiotics are initiated, as disproportional rapid wound breakdown is the typical presentation of necrotizing fasciitis. Even with negative wound cultures, the clinical presentation of postsurgical pyoderma gangrenosum still seems to favour the diagnosis of wound infection. Unfortunately, this misdiagnosis tends to result in prolonged courses of ineffectual antibiotics and surgical debridement that exacerbate and accelerate the problem by perpetuating the pathergic response in skin that was yet unaffected. Biopsies tend to show non-specific neutrophilic inflammation (Fig. 41.9) [36].

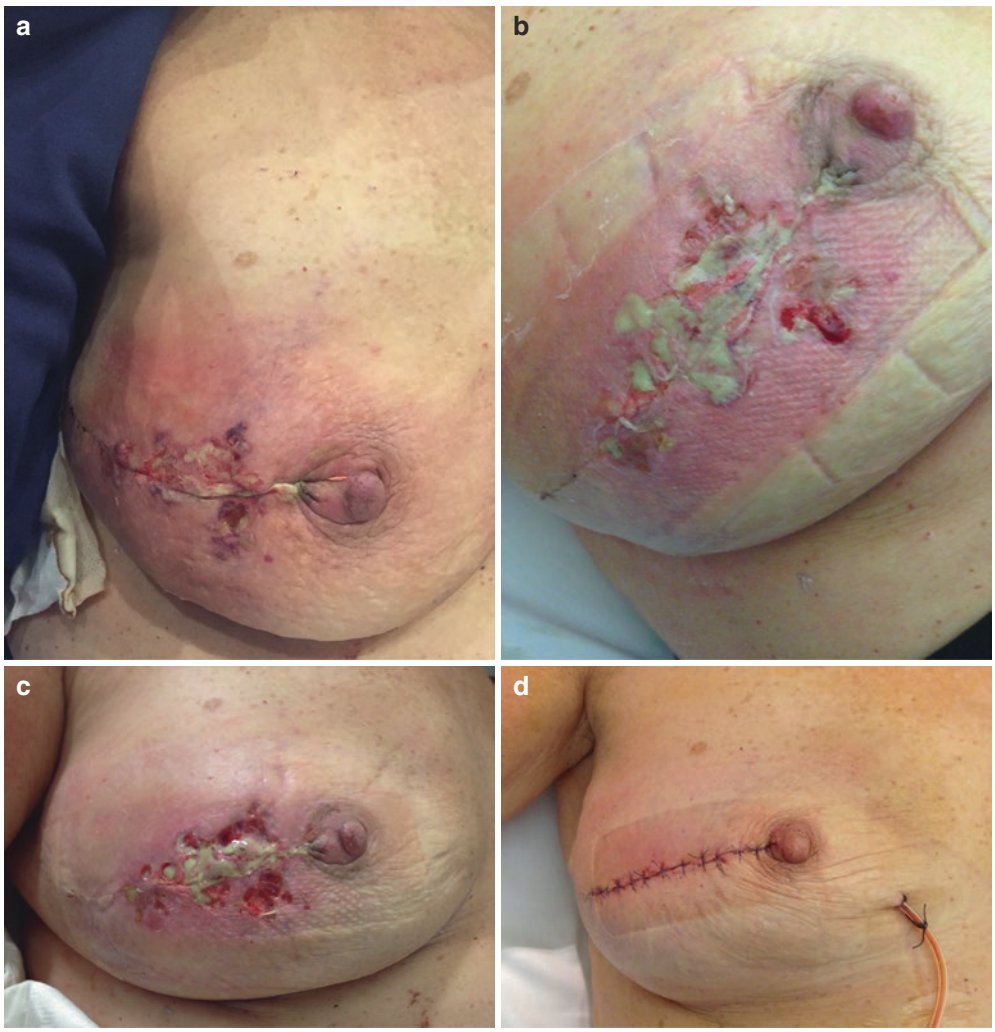

Fig. 41.9 Initial erythema and small punctate ulceration at the surgical site 6 days after right mastectomy and immediate reconstruction with implant (**a**). A wound infection is often diagnosed and antibiotics are initiated. The ulcerations coalesce (**b**—8 days postoperatively and **c**—9 days postoperatively). After systemic corticosteroids therapy, surgical debridement was necessary and successful (**d**—13 days postoperatively)

Awareness of postoperative pyoderma gangrenosum can help early diagnosis and an appropriate management to decrease patient morbidity. Systemic corticosteroids and immunomodulation agents are the first-line therapies; surgical wound debridement and reconstruction (skin grafts) are often necessary to expedite the healing process only if appropriate medical therapy has already initiated [37]. Postoperative pyoderma gangrenosum is a diagnosis of exclusion but should be considered in the differential diagnosis of postoperative wound dehiscence and infection.

41.4 Seroma Formation

Seroma is defined as a fluid collection that can occur both in case of flap reconstruction (at the donor site) and implant reconstruction (around the prostheses).

41.4.1 Managing Seromas at the Donor Site in Flap Reconstruction

Donor site seroma is the most common complication after latissimus dorsi reconstruction reported in the current literature with rates varying from 6% to 95% [38]. Postoperative seroma occurs in a lower percentage of breast reconstruction with abdominal flaps (2–13.5%) [39]. Seroma formation inevitably complicates any extensive surgical dissection and disruption of tissue planes that results in a dead space.

The consequences of developing a donor-site seroma are additional visits to the outpatient clinic for percutaneous aspiration as well as an increased risk of infection.

Several techniques have been described to prevent seroma formation. They minimize the "pocket" created at the donor site. Fibrin glue and quilting sutures are both performed for this purpose [40]. The principle of quilting is to promote flap apposition that facilitates healing. Finally, specially designed supportive garments may reduce seroma formation by applying external pressure on the donor site without jeopardizing the donor vessels.

41.4.2 Managing Seromas Around Breast Implants

Fluid collection around implants is a common event in the immediate postoperative period after drain removal. In case of small amount of serum, it could be asymptomatic, and the excess fluid spontaneously reabsorbs. In case of moderate or large seromas, breast swelling occurs, and percutaneous puncture and drainage are necessary. Ultrasound-guided drainage represents another solution.

The occurrence of late seromas, developing at least 12 months after the most recent breast implant surgery, is a rare event, reported in 0.6% of anatomical silicone form-stable implants [41]. It ranges from 0.4% in primary augmentation patients to 0.9% in reconstruction revision subjects. Late seromas appear as clinically symptomatic breast swelling. They are arousing increased interest in both cosmetic and reconstructive surgery since recent reports describe a possible rare connection between breast implants and anaplastic large cell lymphoma (ALCL), as these tumours often present as late seromas [42, 43, 44, 45].

Late seromas are often related to some sort of trauma and shearing forces between the capsule and textured implants (mechanical theory) [46] or low grade, subclinical infections (e.g. mycobacterium or biofilm—infectious aetiology) [47, 48]. In the vast majority of cases, they appear to be idiopathic, without a clear evidence of infection or malignancy [49].

There are a variety of recently described methods to manage late seromas [50]. Early acquisition of seroma fluid is recommended to rule out infection and malignancy with microbiology and cytology evaluation. The surgeon must decide whether to proceed with percutaneous (ultrasound-guided drainage) versus open therapeutic drainage of the fluid collection. If the decision is surgical drainage, the capsule needs to be inspected to determine whether local biopsy or total capsulectomy is necessary. Implant replacement also needs to be considered. A standard oncological evaluation is recommended to detect associated palpable or radiological masses in the breast or capsule or in the axilla. In fact, although the most common presenting sign of implant-related ALCL is late seroma, in some cases it presents as a mass adherent to the capsule, with or without associated fluid [45].

In our experience, the first approach is percutaneous puncture with culture and cytology. If no suspicious mass is present and fluid drainage resolves the problem, nothing further needs to be done. On the other hand, in case of recurrent seromas, the more definitive and reliable approach appears to be the surgical drainage associated to implant change and possible capsulectomy. In fact, implant-related ALCL tumour cells are usually found both in the fluid and the capsule, but occasionally they are found only in the capsule. In these cases, capsulectomy is essential for diagnosis [45].

41.5 Flap Reconstructions: Donor Side Morbidity

41.5.1 Abdominal Hernia and Bulging, Abdominal Asymmetries

After pedicled TRAM flap reconstruction, parietal complication may occur due to rectus muscle and fascia harvest and relaxation of the fascial suture. Even in cases of DIEP flaps,

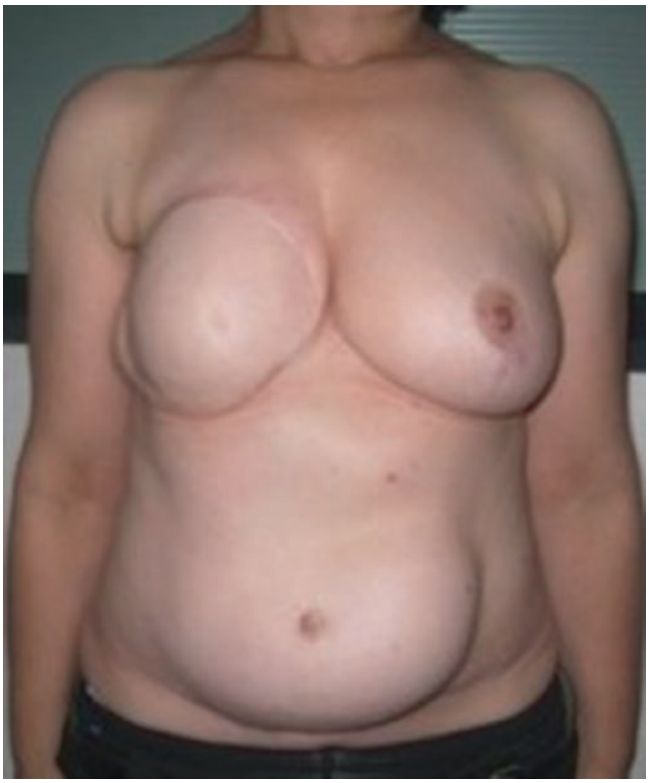

Fig. 41.10 Abdominal hernia after right breast reconstruction with a pedicled TRAM flap

sparing the muscle and the fascia, intramuscular perforator dissection may lead to morbidity [51]. Chang et al. [52] reported an overall hernia/bulge incidence of 5.9% after abdominal-based free flap reconstruction, varying from 3.3 to 9.9% depending on the type of free flap and if these were unilateral or bilateral reconstructions.

Weakness of the abdominal fascia is responsible of laxity and bulging in the infraumbilical region. It can be corrected by plication of the fascia (re-tension) and reinforcement with a preaponeurotic mesh (Fig. 41.10).

Most troublesome are abdominal hernias, which can be localized in the epigastric region (pivot point of the pedicled transfer) or below the umbilical region (anatomical fascia weakness below the arcuate line). They should be treated as if they are symptomatic. A reinforcing mesh is generally used.

41.5.2 Shoulder Function

Latissimus dorsi muscle primarily contributes to shoulder adduction, extension and internal rotation. There is a general consensus that after latissimus dorsi rotation or removal, the actions of synergistic muscles of the shoulder joint compensate for the missing muscle when it comes to the mobility of the shoulder and to carrying out daily activities [53, 54, 55]. Conversely, there are several reports of decreased shoulder strength, but the severity of this loss varies [56].

41.6 Implant Exposure and Extrusion

It is due to insufficient soft tissue or muscular coverage of the implant, and many reasons might be responsible: thin mastectomy flaps or necrosis of the mastectomy flaps and/or deficit in the muscular pocket or wound dehiscence.

It occurs in 0.25–8.3% of implant-based breast reconstructions [57]. In case of actual exposure, the assumption is made that contamination or mild infection is present even though there may be no clinical evidence of infection. Therefore, all exposed devices are treated accordingly. Salvage attempts and decision-making process is based on the severity of local infection and on the availability of soft tissue coverage. Thus, initially the patient is covered with antibiotics, the device is removed, a capsulectomy is performed, the pocket is curetted, a new device is placed and closure is performed with local or distant tissue [17]. If sufficient local tissue is present, local tissue rearrangement may be adequate. If local tissue is not adequate, a distant flap should be used for coverage. If a flap has been used in the initial procedure before implant exposure and this distant tissue is unavailable for a salvage procedure, the implant should be removed in favour of a delayed reinsertion (Fig. 41.11).

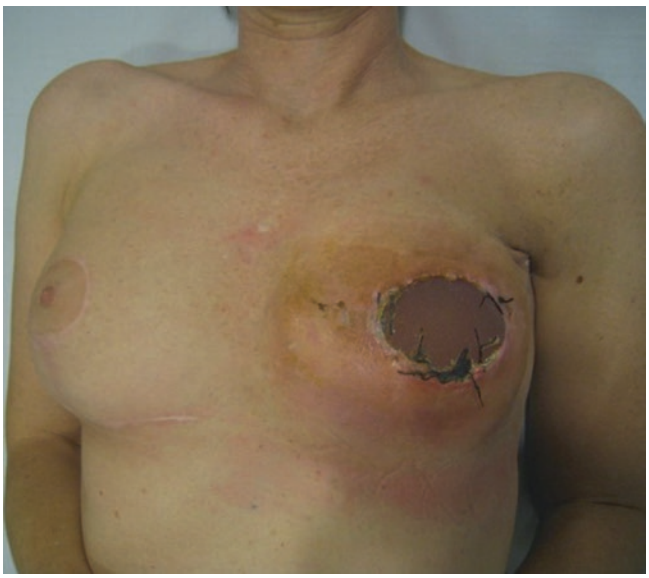

Fig. 41.11 Left implant exposure and extrusion after necrosis of the mastectomy flaps and wound dehiscence

41.7 Implant Rupture

It is defined as a gap in implant envelope leading to silicone gel or saline diffusion outside the implant itself. Saline prostheses are almost abandoned nowadays; in case of rupture, the implant deflates, and it is clinically evident. Conversely, the majority of implants on the market are silicone ones composed of a textured silicone elastomer shell and filled with cohesive silicone gel. Cohesive gel is formed by increasing the number of cross-links between gel molecules, with results in form-stable implant less likely to fold or collapse.

Rupture occurs as a result of biochemical degradation of silicone, physical trauma to the elastomer at the time of implantation or as a result of mechanical injuries during mammograms, closed capsulotomies or accidents. Intracapsular rupture is defined by the presence of silicone outside the implant shell and within the intact fibrous capsule. Extracapsular rupture is defined by the presence of silicone into surrounding tissues and lymph nodes.

The incidence of implant rupture widely varies in the literature [58, 59], and its prevalence increases over time. It depends on the type and generation of implants, different detection methods, mean implant life span and different follow-up period. In 2015, 10-year results from the Natrelle 410 anatomical form-stable silicone implants have been published. The overall rupture rate (suspected and confirmed) in those patients who underwent bi-annual MRI to screen for silent implant rupture is 9.7% of implants at a 10-year follow-up [41]. Rupture rates are even lower using Mentor MemoryShape implants, but results at a 10-year follow-up are not yet available, and patients have been screened with MRI at 8 years [60, 61, 62].

The majority of silicone implant ruptures are asymptomatic and are detected during routine follow-up ultrasounds. In case of suspicious images with ultrasound, MRI is recommended. MRI is the most accurate technique to evaluate implants integrity. Its sensitivity for rupture is between 80% and 90% and its specificity between 90% and 98% [63].

Explantation is the gold standard, with the removal of the capsule to include eventual silicone residuals.

41.8 Capsular Contracture

The pathologic process of capsular contracture manifests from excessive peri-implant fibrosis or capsular formation beyond the normal state. Clinically, it can manifest as pain, hardening of the breast and distortion of the reconstructed breast. The rate and risk of capsular contracture remain controversial. It increases over time [64], and it is reported as baker III and IV in 14.5% of the patients at 10 years after reconstruction with the Natrelle 410 anatomical form-stable silicone breast implant [41]. A meta-analysis (level Ib evidence) demonstrated that textured implant shells clearly reduce the risk of contracture for subglandular implants [65]. Review of randomized controlled trials found a significantly increased risk of contracture for smooth subglandular implants [66]. Its incidence is significantly higher in cases of irradiated beasts [67].

Despite advances in breast implant surgery, it is the most frequent cause for implant revision, and capsulotomy and capsulectomy represent the standard treatments.

The exact cause for capsular contracture has yet to be determined. Several theories on the pathomechanism and origin of capsular contracture have been suggested. These theories underpin the pivotal role of an inflammatory reaction, which leads to induction of fibrosis and shrinking of the capsule. A non-specific inflammatory process directed against silicone and periprosthetic bacterial contamination is considered to be the primary pathogenic mechanism leading to excessive local inflammation [68]. Therefore, treatment strategies also include the use of targeted inhibitory molecules as the leukotriene inhibitor zafirlukast to affect capsule formation [69, 70]. These drugs are also used in the prophylaxis of contracture. Implant insertion with a funnel may also decrease capsular contracture reducing skin contact and potential contamination of the implant pocket with skin flora [71].

Finally, more recently, acellular dermal matrices have been proposed to decrease the incidence of capsular contracture in implant-based reconstruction. They are hypothesized to block the inflammatory process suspected to be the trigger in the pathogenesis of capsular contracture, and several animal models support this statement [72]. In the clinical setting, the higher level of evidence (level III) is represented by a study comparing capsular contracture rates in a cohort of women who had acellular matrix-assisted implant reconstruction against a cohort who underwent standard implant reconstruction [73]. This study concluded that acellular dermal matrix is associated with less capsular contracture.

41.9 Systemic Complications

41.9.1 Pneumothorax

Pneumothorax remains a serious although rare complication of breast reconstruction. Most cases in the plastic surgery literature relate to breast augmentation, but they are described also in case of tissue expander/prosthesis placement [74] and autologous reconstruction [75]. The American National Incidence of pneumothorax after expander reconstruction is 0.55% per patient [76].

When pneumothorax occurs, it may cause significant morbidity. Delay of diagnosis may be fatal as the patient can quickly become hemodynamically unstable.

Clinically it can be classified as spontaneous (no obvious precipitating cause present) or traumatic. In patients with breast implant, it may be a direct complication of surgery (pleural damage) or secondary to pulmonary blebs. The mechanism of damage to the parietal pleura during surgery may include intraoperative pleura laceration during capsulectomy or creating a new muscular pocket, needle puncture for anaesthetic infiltration and pleural rupture due to high anaesthetic ventilation pressure. Falling SpO_2 levels despite oxygen supplementation generally occur. Chest auscultation reveals reduced air entry on the affected side, and chest X-ray can confirm the diagnosis. Chest drain insertion is required for successful treatment, determining good reinflation of the lung seen on check chest X-ray.

41.9.2 Pulmonary Embolism

Incidence of symptomatic pulmonary thromboembolism postoperatively after breast reconstruction is low, and it is reported in 0.7% of the patients after pedicled TRAM reconstructions [39]. Unfortunately, however, this number underestimates the true value because asymptomatic events may not have been identified. These accidents may present a dangerous and dreaded complication, even towards mortal pulmonary embolism.

Both latissimus dorsi and pedicled TRAM are associated with risk factors for deep vein thrombosis and pulmonary embolism including underlying malignancy (immediate reconstructions), operation time and transient immobilization in the postoperative period [77]. Abdominal flaps, however, have been thought to decrease venous return of the pelvis and lower extremities in superficial veins, increasing the risk of deep vein thrombosis and subsequent pulmonary embolism. In addition, an abdominal flap generally has a longer period until full ambulation in comparison with latissimus dorsi reconstruction [78]. This further supports the importance of proper deep vein thrombosis prophylaxis. The combination of the type of reconstruction (immediate vs. delayed procedures, abdominal flaps, dermolipectomy) and the predisposing individual patient risk factors (age, obesity, varicose veins, venous thromboembolism history, coagulation disorders) defines the level of thromboembolic risk that can be mild, moderate or high [79]. Prevention is based on general guidelines (early mobilization) and, at every level of potential risk, on the use of low molecular weight heparin and/or wearing antithrombosis stockings. If thromboembolic complications occur, their surveillance with duplex venous scanning of lower limbs is recommended at an early stage as well as appropriate systemic treatment to avoid evolution towards pulmonary embolism.

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Part VII

Medical Oncology

Adjuvant Systemic Therapies by Subtypes: Guidelines

42

Antonella Palazzo and Marco Colleoni

42.1 Introduction

The adjuvant treatment of breast cancer involves a multidisciplinary approach including surgery followed by medical treatments and radiotherapy as clinically indicated. Adjuvant systemic treatment aims to reduce the risk of breast cancer relapse, in terms of locoregional and distant events, and to prolong survival. Adjuvant treatment options for primary breast cancer include chemotherapy, endocrine therapy, biological therapy, or combination of these. Decision on adjuvant systemic therapy should be balanced on the risk of relapse, patients' comorbidities and preferences, and the potential absolute treatment benefit. It should be tailored according to tumor burden and biological behavior of cancer.

Invasive breast cancer has been historically classified according to histopathological parameters such as histomorphologic features, tumor size, nodal involvement, and presence of metastases. Recently, it has been more evident that breast cancer heterogeneity reflects a high complexity of molecular composition with different subtypes varying in their characteristics and natural history.

Perou et al. described different gene expression patterns of breast cancer. Through an extensive genomic analysis of breast cancers, authors identified four molecular subtypes (luminal A, luminal B, HER2 enriched, and basal-like) with different prognoses [1, 2].

In order to better define treatment decision, breast cancer subtypes can be defined by the use of multiparameter molecular tests such as MammaPrint (Agendia BV, Amsterdam, Netherlands), Oncotype DX (Genomic Health Inc., USA), PAM50 risk of recurrence (ROR) score (Prosigna Breast Cancer Prognostic Gene Signature Assay; NanoString Technologies, Seattle, WA), EndoPredict (EP) assay, and Breast Cancer Index [3–7]. Although in selected areas of the world multigene assays are readily available, due to their costs, their use is not possible in many countries. Surrogate pathological classification of subtypes has been studied by immunohistochemical (IHC) determination of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor (HER2) status, and proliferative index (Ki-67 index). Even if similar to the intrinsic subtypes, the pathological classification is not entirely the same. About 72% of triple-negative tumors are basal-like, 9% HER2 enriched, 6% luminal B, and 5% luminal A [8].

The future of breast cancer treatment is based on developing regimens that provide the greatest clinical benefit with lower side effects. Molecular diagnostic tests can provide prognostic information about cancer in its early stages. International guidelines recognize the assay's ability to predict a patient's risk of recurrence or benefit from chemotherapy. The assays are all considered usefully prognostic for years 1–5. PAM50 ROR® score is considered to be clearly prognostic beyond 5 years, whereas only Oncotype DX showed its value in predicting the usefulness of chemotherapy [9, 10, 11, 12].

According to the widely used immunohistochemistry classification of subtypes, adjuvant treatment guidelines will be examined for hormone receptor-positive HER2-negative disease that include two distinct prognostic groups, "luminal A-like" and "luminal B-like," based on Ki-67 expression level or on the expression of PgR; the "HER2-positive" group (which expresses HER2 by IHC or amplification detected by fluoresce in situ hybridization [FISH]) and the "triple-negative" group (which is negative for ER, PgR, and HER2).

42.2 Luminal-Like A and Luminal-Like B HER2-Negative Subtypes

The luminal-like subtypes are characterized by tumors that are clinically described as ER positive and a relatively high expression of many genes expressed by breast luminal cells.

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The Cancer Genome Atlas Research Network described ER-positive (luminal) breast cancers as the most heterogeneous in terms of gene expression, mutations, and copy number changes. The heterogeneity is not only for the luminal genes expression. In fact, luminal subtypes were further distinguished into at least two subgroups on the basis of their "proliferation cluster": luminal A and luminal B tumors. The proliferation cluster is a group of genes whose levels of expression correlate with cellular proliferation rates. The TP53 pathway is differently activated in this subtype, with a low TP53 mutation frequency in luminal A (12%) and a higher frequency in luminal B (29%). Other differences between luminal A and B included the hyper-activation of some transcriptional activity as c-MYC in luminal B breast cancer. This cluster also included the genes encoding the widely used immunohistochemical markers of cell proliferation as Ki-67 index [13].

The luminal A subtype of breast cancer has the best prognosis among all subtypes [2]. European guidelines define the clinicopathological surrogate of luminal A-like subtypes as those tumors with high estrogen receptor expression; low proliferation index; high progesterone receptor expression (with a suggested cutoff value of 20%); negative HER2 and a low tumor burden, meaning a lower or absent nodal involvement (N 0–3); and smaller T size (T1 or T2) [9, 12].

All luminal cancers should be treated with endocrine therapy (ET). ET is indicated in all patients with detectable ER expression (defined as ≥1% of invasive cancer cells) independently of the use of chemotherapy and/or targeted therapy. Hormonal therapies include selective estrogen receptor modulators (SERMs), such as tamoxifen, and aromatase inhibitors. The choice of agent is primarily determined by the patient's menopausal status (see Chap. 4). Other factors include side effect profiles.

It is among those luminal breast cancer patients that clinicians express highest uncertainty about optimal treatment and chemotherapy indications, as clinicians seek to avoid overtreatment and undertreatment. Results from two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer patients showed no benefit of chemotherapy in the subgroup with high ER receptor, negative HER2, and low proliferation [14].

Moreover, the first retrospective studies with generation multigene signatures, as Oncotype DX, also showed that among patients with ER-positive breast cancer, there is no benefit of chemotherapy treatment for the low proliferative tumors. The 21-gene recurrence score (RS) assay (Oncotype DX; Genomic Health Inc., Redwood City, CA) quantifies risk of distant recurrence in patients with node-negative, estrogen receptor (ER)-positive breast cancers and has been validated retrospectively in two independent datasets. In the NSABP-B20 trial, patients with ER-positive, node-negative breast cancer were randomized to receive tamoxifen or tamoxifen plus a first-generation chemotherapy regimen (cyclophosphamide, methotrexate, and 5-fluorouracil). Chemotherapy showed a clinical benefit only in patients with high recurrence score (RS > 31) [15].

Albain et al. investigated whether the RS was prognostic in patients enrolled into the phase III SWOG-8814 trial, in which postmenopausal women with node-positive, ER-positive breast cancers were randomized to receive tamoxifen alone or tamoxifen plus a second-generation chemotherapy regimen (cyclophosphamide, adriamycin, and fluorouracil [CAF]). The study showed no benefit of CAF in patients with a low RS (score < 18; log-rank $p = 0.97$; HR 1.02, 0.54–1.93), while an improvement in DFS was seen for those with a high RS (log-rank $p = 0.033$), after adjustment for number of positive nodes [16]. This trial demonstrated that patients with high RS will benefit from chemotherapy, whereas those with low RS did not, irrespective of nodal burden.

Pending results from ongoing trials, the TAILORx trial (Trial Assigning IndividuaLized Options for Treatment) and the MINDACT trial (Microarray In Node-negative and 1–3 positive lymph node Disease may Avoid ChemoTherapy), no prospective data are available about the use of biological subtypes as eligibility criteria to adjuvant study treatment.

Recently partial results from TAILORx trial have been published with an analysis of the women in the lowest-risk group [17]. The findings showed that for the cohort of patients who had a recurrence score of 0–10 and were assigned to receive endocrine therapy alone without chemotherapy, at 5 years, in this patient population, the rate of distant relapse-free survival was 99.3%, of invasive disease-free survival was 93.8%, and of overall survival was 98.0%. These results provide prospective evidence that the gene expression test can identify women with a low risk of recurrence who can be spared chemotherapy.

On the other hand, the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis from analysis of 123 randomized trials showed that taxane-plus-anthracycline-based regimens improve substantially the long-term, relapse-free, and overall survival independently by age, nodal status, tumor diameter, differentiation, and subgroup [18].

According to the available evidence, the National Comprehensive Cancer Network (NCCN) guidelines indicate to add chemotherapy to endocrine therapy for ER-positive HER2-negative node-positive disease (one or more ipsilateral lymph nodes) and for node-negative disease both for intermediate and higher recurrence score risk subgroups of patient tested by Oncotype DX test [11]. The 2015 St. Gallen Consensus report and European Society for Medical Oncology (ESMO) guidelines recommend to consider chemotherapy other than endocrine therapy not only in patients with multiparameter molecular marker at

"unfavorable prognosis" if available but also looking at clinicopathological surrogate classification for luminal-like A disease if four or more nodes are involved or if there are characteristics of luminal-like B disease, such as lower ER/PgR with clearly high Ki-67, more extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, and larger T size (T3) [9, 12].

42.3 Luminal B-Like HER2-Positive Subtypes and HER2-Enriched Subtypes

The human epidermal growth factor receptor 2 (*HER2*) gene (also referred to as *ERBB2*) is amplified and/or overexpressed in approximately 15–20% of primary breast cancers.

Gene expression studies revealed that HER2-positive subtypes were characterized by an expression of several genes in HER2 amplicon and a significant frequency of TP53 mutation (71% of cases). More extensive studies with 50-gene test "PAM50" [7, 19] depicted HER2-enriched subtype as clinically HER2 positive, with high proliferation index, low expression of luminal genes, and lack of expression of basal cluster. The surrogate immunohistochemical markers to distinguish this subtype are hormone receptor-negative (ER and PgR) and HER2-positive breast cancer.

HER2-positive breast cancer could also express hormone receptors. This histopathological subtype is similar to the genome intrinsic subtype known as luminal B HER2-positive (hormone receptor positive and HER2 positive).

Overexpression of the ERBB2 oncoprotein is a well-known poor prognostic factor, and studies on clinical outcome of different subtypes revealed also that the basal-like and the HER2-positive subgroups were those with the shortest survival and relapse-free survival [2].

Trastuzumab is a recombinant humanized monoclonal antibody. The addition of trastuzumab to conventional chemotherapy in breast cancer patients overexpressing HER2 dramatically improves disease-free and overall survival in the adjuvant setting, with a 10% absolute improvement in disease-free survival (DFS) and 9% increase in 10-year overall survival (OS) [20–22].

Adjuvant treatment guidelines are unanimous to indicate chemotherapy with anthracyclines followed by a taxane-containing regimen concurrent to trastuzumab for patients with stages 2 and 3. Some specific considerations among guidelines were developed for stage 1 disease. For pT1b (tumor diameter, 0.6–1 cm) and pT1c (tumor diameter, 1.1–2 cm) tumor, chemotherapy associated to trastuzumab is also recommended [23]. The preferred chemotherapy regimen remains anthracyclines and taxane based specially for pT1c tumors, but also regimen avoiding anthracycline (paclitaxel combined to 1 year of trastuzumab) is a considerable option for pT1b tumors, based on the excellent outcome recently showed [24]. In selected cases with stage 1 disease and a very low risk, as pT1a tumor (tumor diameter 1.1–5 mm), chemotherapy and trastuzumab can be omitted.

Luminal B HER2-positive disease, differently from HER2-enriched subtype (non-luminal), requires endocrine therapy in addition to chemotherapy plus trastuzumab. Endocrine therapy should be given sequentially to chemotherapy, concomitantly to trastuzumab, and the choice of endocrine therapy should be always suggested by patient's menopausal status. No randomized trials exist to support endocrine therapy plus trastuzumab avoiding chemotherapy in this tumor subtype.

Only in selected cases of contraindications to chemotherapy or patient refusal might the combination of targeted agents (endocrine therapy and trastuzumab) be offered [12].

Based on adjuvant trials, the administration of trastuzumab should be avoided concomitantly to anthracyclines due to its cardiotoxicity (not routinely recommended outside of clinical trials) [20–22, 25], but it may be safely used concomitantly to taxane and also during radiotherapy, when indicated. It has been in fact demonstrated that trastuzumab concomitantly to taxane regimen is more effective than sequential use [21].

Standard duration of trastuzumab is 1 year according to literature data [20–22, 26, 27, 28].

No statistically significant evidence of superiority in terms of disease-free survival was observed combining dual anti-HER2 therapy including trastuzumab and lapatinib, an oral dual inhibitor of epidermal growth factor receptor (EGFR) and HER2 tyrosine kinases [29].

42.4 Triple-Negative Subtype

Among breast cancer subtypes, gene expression array study identified the basal epithelial cells that expressed a characteristic gene cluster including keratin 5, keratin 17, integrin beta 4, and laminin. These tumors also showed a lack of ER and most of the other genes that were usually co-expressed with estrogen receptors [1].

Basal phenotype is defined as negative for hormone receptors and HER2 and positive for cytokeratin 5/6 or epidermal growth factor receptor; it represents a different clinical entity associated to highest TP53 mutations and shorter survival times with a 5-year survival rate of 79.0% (70.8–85.3) and a 73.5% disease-free survival at 5 years (65.0–80.5) [2, 30].

Randomized controlled trials and retrospective studies evaluating the correlation between chemotherapy benefit and ER status suggest that the magnitude of the benefit of chemotherapy is large in patients with ER-negative subtypes

[18, 31]. Due to the absence of known specific therapeutic target, chemotherapy is the standard indication of the international guidelines favoring anthracyclines and taxane-based regimens [9, 11, 13].

Classical cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) is reported as effective for the treatment of TNBC and may still be used in selected cases [14, 32].

Controversies exist about the use of anthracycline and taxane dose-dense schedules (with granulocyte colony-stimulating factor support) [33, 9, 11, 13] although this treatment can be regarded as a reasonable option, particularly in highly proliferative tumors.

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Primary Systemic Therapies: Guidelines

43

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43.1 General Principles

The idea of prioritizing the systemic treatment before surgery was first developed based on the hypothesis to reduce the risk of progress to metastatic disease if the disseminated tumor cells are rapidly killed. However, further studies did not confirm this assumption. A meta-analysis of nine randomized trials found no differences between neoadjuvant and adjuvant treatments according to death, disease progression, and distant disease recurrence [1]. Two big randomized phase III trials, the NSABP B-18 and the NSABP B-27 study, did not show any difference in disease-free survival (DFS) and overall survival (OS) between preoperative and postoperative chemotherapy. Only a trend in favor of preoperative chemotherapy for DFS and OS in women younger than 50 years was observed in the NSABP B-18 study [2].

Three main reasons for choosing neoadjuvant therapy can be identified: to improve the surgical outcome, to determine the response to preoperative treatment, and to obtain long-term disease-free survival. In locally advanced breast cancer, the major goal is to facilitate surgical options and in patients with operable breast cancer candidate for adjuvant chemotherapy, to obtain freedom from disease [3].

Recommendations

1. The neoadjuvant chemotherapy (NACT) should be applied only if invasive breast cancer is diagnosed.
2. Preoperative treatment should be considered in women with locally advanced breast cancer or large operable tumors.
3. The same regimen recommended in the adjuvant setting could be used in the neoadjuvant setting [4] (Table 43.1).
4. The addition of taxanes to anthracycline-based NACT should be used to improve the pathologic complete response (pCR) and DFS and decreases the incidence of local recurrence [4].
5. Dose-dense NACT has been shown to improve pCR, disease-free survival, and overall survival compared to standard dose chemotherapy [11]; notably, pCR has a potential surrogate value for survival in trials comparing dose-dense regimens versus standard regimens [17]. As it is less tolerated compared to standard treatment, dose-dense chemotherapy should only be considered as investigational.
6. The full planned treatment should be completed before surgery to increase the chance of pCR [18] (Table 43.2).
7. At least six cycles equivalent to at least 18 weeks of NACT should be administered [7, 23].
8. Following a full course of NACT, no additional postoperative adjuvant chemotherapy is recommended, whether pCR was achieved or not [24]. However, preliminary data suggest an improvement in DFS for patients without pCR receiving six cycles of capecitabine [25].
9. If no response is observed in an inoperable tumor or the disease progressed under treatment, alternative chemotherapy regimens should be carefully considered. Available results deriving from clinical trials failed to show additional benefit especially among patients with hormone receptor-positive tumors who did not respond to standard initial NACT, switching to a non-cross-resistant regimen [26, 27]. In this scenario, preoperative radiotherapy should also be taken into account [4] (Table 43.2).

43.2 HER2-Positive Breast Cancer

Trastuzumab is a monoclonal antibody directed against the sub-domain IV, a juxta-membrane region of HER2's extracellular domain. It exerts its effect by inhibiting the ligand-independent signaling and by blocking HER2 cleavage. Buzdar et al. firstly demonstrated that the addition of trastuzumab to anthracyclines-taxane-based chemotherapy significantly increased the pCR rate. Patients were treated

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Table 43.1 Recommended regimen in the neoadjuvant setting

Regimens	References
Standard regimens used in the adjuvant setting with a duration of at least 18 weeks	Kaufmann et al. [5]
AC or EC → D q3w or P q1w	Rastogi et al. [2]; von Minckwitz et al. [6]
DAC	Von Minckwitz et al. [7]
AP → CMF	Gianni et al. [8]
Taxane followed by anthracycline sequence	Bines et al. [9]; Earl et al. [10]
Dose-dense regimen (e.g., E-P-CMF, E-P-C)	Untch et al. [11, 12]
Platinum in TNBC	Von Minckwitz et al. [13]; Sikov et al. [14]; Petrelli et al. [15]
Platinum in case of family history of breast/ovarian cancer or BRCA alteration	Byrski et al. [16]

AC doxorubicin, cyclophosphamide, *EC* epirubicin, cyclophosphamide, *DAC* docetaxel, doxorubicin, cyclophosphamide, *AP* doxorubicin, paclitaxel, *CMF* cyclophosphamide, methotrexate, 5-fluorouracile

Table 43.2 Procedures in case of early response, no change or progressive disease during neoadjuvant chemotherapy

Early response	References
Complete all chemotherapy before surgery	von Minckwitz et al. [18]; von Minckwitz et al. [7]; Kaufmann et al. [5]
In case of response after two cycles of DAC in HR-positive breast cancer, consider eight instead of six cycles of DAC	von Minckwitz et al. [19]
No change	
Completion of NACT, followed by surgery	von Minckwitz et al. [7]; Kaufmann et al. [5]
Continuation of NACT with non-cross-resistant regimen	Bear et al. [20, 21]; von Minckwitz et al. [19]
Progressive disease	
Stop of NACT and immediate surgery or radiotherapy	Kaufmann et al. [5]
Additional adjuvant chemotherapy with non-cross-resistant regimen	Mittendorf et al. [22]

DAC docetaxel, doxorubicin, cyclophosphamide

with 4 cycles of paclitaxel followed by 4 cycles of FEC-75 (5-fluorouracile, epirubicin, cyclophosphamide); trastuzumab was given simultaneously with a weekly schedule. The pCR rate was more than double in the trastuzumab arm (65.2% vs. 26.0%; $p = 0.016$) [28], with long-term follow-up not showing new safety concerns [29]. The HER2-positive cohort of the GeparQuattro trial showed a pCR in 31.7% of tumors [30]. Moreover, the NOAH trial showed a higher DFS when trastuzumab was added to a neoadjuvant regimen consisting of doxorubicin and paclitaxel followed by paclitaxel or CMF (cyclophosphamide, methotrexate, and 5-fluorouracile) (5-year DFS 58% vs. 43%; HR = 0.64; $p = 0.016$) [31, 32]. The following studies investigated the effect of different anti-HER2 treatments on pCR as well as the combination of targeted agents. Lapatinib is an inhibitor of the intracellular tyrosine kinase domains of both EGFR and of HER2 receptors. In the GeparQuinto trial, patients receiving trastuzumab as part of standard anthracycline-taxane-based chemotherapy experienced higher pCR compared to patients receiving only lapatinib (30.3% vs. 22.7%; $p = 0.04$) [33]. Similar results emerged from the NeoALTTO trial [34]. Moreover, patients receiving both anti-HER2 agents experienced a significantly higher pCR rate compared to trastuzumab alone (51.3% vs. 29.5%; $p < 0.01$). In the NSABP B-41 study, patients were randomized to receive 4 cycles of doxorubicin and cyclophosphamide followed by 4 cycles of weekly paclitaxel. Concurrently with weekly paclitaxel, patients received either trastuzumab weekly, lapatinib daily, or trastuzumab plus lapatinib until surgery. Substitution of lapatinib for trastuzumab in combination with chemotherapy resulted in similarly high percentages of pCR (53.2% vs. 52.5%; $p = 0.985$). Combined HER2-targeted therapy did not increase the pCR rate compared to the single-agents alone [35]. Several other studies showed a lower pCR rate when lapatinib was used as single agent as part of NACT [36–38].

Pertuzumab is a monoclonal antibody directed at the dimerization domain of HER2 that exerts its effect by inhibiting the ligand-dependent signaling, particularly between HER2 and HER3. The NeoSphere trial firstly demonstrated the achievement of a higher pCR rate with the dual anti-HER2 blockade pertuzumab and trastuzumab added to docetaxel compared to trastuzumab or pertuzumab alone. Only marginal benefit was seen when the two agents were administered alone without the chemotherapy backbone [39]. In the pertuzumab/trastuzumab and pertuzumab/docetaxel arms, respectively, 3-year survival rates were 88% vs. 84% for DFS and 81% vs. 82% for PFS. Across all four treatment arms, for all patients who achieved pCR (ypT0/is ypN0) versus all patients who did not achieve pCR, the HR for DFS was 0.68 (95% CI, 0.36–1.26) and the HR for PFS was 0.54 (95% CI, 0.29–1.00). The TRYPHAENA trial confirmed the efficacy of the double anti-HER2 blockade in particular when given together with docetaxel and carboplatin. Importantly, a lower rate of symptomatic left ventricular systolic dysfunction was reported [40].

Given these data and in conjunction with the high efficacy of pertuzumab in the metastatic breast cancer setting, pertuzumab in combination with trastuzumab and docetaxel gained accelerated approval from the Food and Drug Administration (FDA) in 2012 for the use in the neoadjuvant setting, as well as European Medicines Agency (EMA) authorization in 2015.

Recommendations

1. Women with HER2-positive breast cancer should receive trastuzumab as part of neoadjuvant chemotherapy in order to increase the chance of achieving a pCR.
2. Pertuzumab in combination with trastuzumab and docetaxel is a valid option. Due to the limited information on its effect on survival, the use might be restricted to more advanced tumors (e.g., tumors greater than 5 cm or nodal involvement).
3. The use of lapatinib as a single agent is not approved. Given the conflicting results available and its more unfavorable toxicity profile, the use of lapatinib in combination with trastuzumab in the neoadjuvant setting is not recommended.

43.3 Triple-Negative Breast Cancer

Given the significant prognostic impact of pCR on outcome [41], several agents have been under investigation in triple-negative breast cancer (TNBC), in order to increase the pCR rate. Whether or not carboplatin should routinely be administered as part of standard NACT in patients with TNBC is still a matter of debate. In the GeparSixto study, the addition of neoadjuvant carboplatin to a regimen of a taxane and anthracycline significantly increased the proportion of TNBC patients achieving a pCR (with carboplatin, 53.2%, 95% CI 54.4–60.9; without carboplatin, 36.9%, 95% CI 29.4–44.5; p = 0.005) [13]. Moreover, patients with TNBC were found to have homologous recombination-deficient tumors in 70.5%, which has been shown to be independently predictive of high pCR rates following neoadjuvant treatment with paclitaxel and liposomal doxorubicin with or without carboplatin [42]. Interestingly carboplatin induces a significantly improved disease-free survival in patients with TNBC (with vs. without carboplatin, 85.8% vs. 76.1%; HR = 0.56 [95% CI 0.33–0.96]; p = 0.035) but not in patients with HER2-positive disease (with vs. without carboplatin, 86.7% vs. 83.4%; HR = 1.33 [95% CI 0.71–2.48]; p = 0.372) (interaction test p = 0.046) [43].

The CALGB 40603 evaluated the impact of adding carboplatin and/or bevacizumab to standard anthracycline-taxane-based chemotherapy. The addition of either carboplatin (60% vs. 44%; $p < 0.001$) or bevacizumab (59% vs. 48%; $p < 0.001$) significantly increased pCR defined as ypT0/is, whereas carboplatin only (54% vs. 41%; p = 0.003) significantly increased pCR in breast and nodes (ypT0/is ypN0) [14]. Despite significantly higher pCR rates, neither carboplatin nor bevacizumab has yet been shown to improve relapse-free survival or OS when administered as part of neoadjuvant therapy in TNBC [44].

Further trials investigated the role of bevacizumab in TNBC. In all of them, the anti-VEGF therapy was able to increase the pCR rate compared to anthracycline-taxane-based chemotherapy alone [45–47]. The GeparQuinto study as well as two big adjuvant trials did not show an improvement in survival with bevacizumab [48–50]. In contrast, the NSABP B-40 trial reported a significant increase in survival with the addition of neoadjuvant plus adjuvant bevacizumab [51]. In the GeparSepto study, nab-paclitaxel increased significantly the pCR rate compared to paclitaxel (38.0% vs. 29.0%; $p < 0.001$), especially in patients with triple-negative tumors (48.2% vs. 25.7%; $p < 0.001$) [52]. The ADAPT TN trial suggested high efficacy and favorable toxicity of 12 weeks therapy with nab-paclitaxel and carboplatin compared to nab-paclitaxel and gemcitabine in TNBC (49.2% vs. 25%; p = 0.006) [53].

Recommendations

1. A sequential regimen of anthracycline-taxane-based chemotherapy should be applied to the majority of patients.
2. Carboplatin and nab-paclitaxel seem to increase responses in patients with TNBC, the former at the cost of major hematological toxicities and discontinuations and the latter of a higher rate of sensory neuropathies. Ambiguous results have been reported for the effect of carboplatin on survival. Therefore, the decision of adding these agents to standard NACT should be based on the individual basis weighing risks and benefits for the patient.
3. So far, there is no role for bevacizumab in the neoadjuvant setting in patients with HER2-negative disease.

43.4 Neoadjuvant Chemotherapy in Luminal B-Like Tumors

Hormone receptor (HR)-positive/HER2-negative breast cancer even with a higher proliferation rate (luminal B-like) has a lower chemosensitivity compared to the HER2-positive or triple-negative subtype and needs longer treatment in order to increase the probability to achieve a pCR [54]. For luminal B-like high-risk breast cancer, chemotherapy followed by hormone therapy is recommended for the majority of cases (conventionally dosed AT-based chemotherapy; dose-dense chemotherapy in case of high tumor burden). All patients with luminal B-like HER2-positive breast cancer should receive chemotherapy, anti-HER2 treatment, and hormone therapy [24].

43.5 Neoadjuvant Endocrine Therapy

The neoadjuvant endocrine therapy is an alternative approach in women with luminal A-like breast cancer, in particular when high levels of estrogen receptor are present. Invasive

lobular carcinomas show significantly lower pCR rates after NACT compared to the other histological tumor types, mainly due to their HR positivity [55]. Several trials show the superiority of the aromatase inhibitors anastrozole and letrozole over tamoxifen in terms of objective response and breast conserving surgery [56–58]. In premenopausal patients, the use of goserelin together with anastrozole leads to a higher rate of partial or complete response compared to goserelin plus tamoxifen (70.4% vs. 50.5%; estimated difference between groups 19.9% [95% CI 6.5–33.3]; $p = 0.004$) [59].

Compared to neoadjuvant chemotherapy, endocrine treatment needs more time to show a response and to achieve a pCR. Therefore, treatment should continue for at least 4–8 months or until maximum response [60]. Moreover, considering the time needed to observe a decrease in tumor size and the lower pCR rate, a careful patient selection is mandatory. Given the antiproliferative effect of hormone therapy, Ki67 has been widely evaluated as an early predictor of response. It has been demonstrated that levels of Ki67 during treatment are more related to long-term prognosis compared to pretreatment levels and change in Ki67 is accepted as a validated end point for comparing endocrine agents in the neoadjuvant scenario. Patients who failed to obtain an early reduction in Ki67 levels need to be considered for an alternative approach [61, 62].

Recommendations

1. In patients with luminal A-like breast cancer, neoadjuvant endocrine therapy should be considered if a rapid onset of response is not necessary.
2. Aromatase inhibitors are the agents of choice in postmenopausal patients, and in premenopausal patients, GnRH analogues might be added.
3. Treatment should be prolonged until achievement of maximum response.
4. Ki67 should be used to identify early responders. If no Ki67 reduction is observed, other agents should be considered.

43.6 pCR and Outcome

The relationship between pCR and outcome has been widely investigated. Two large meta-analyses tried to define if pCR could be used as surrogate end point for survival. The German Breast Group investigated the association between pCR and outcome in 6377 patients with primary breast cancer receiving neoadjuvant anthracycline-taxane-based chemotherapy in seven randomized trials. Overall, patients with no residual disease in breast and nodes (ypT0 ypN0) experienced a better DFS and OS, but the impact of pCR on outcome broadly varies among subgroups. In patients with less aggressive tumors (low proliferation, lobular, grade 1, hormone receptor positive), pCR was not predictive for OS and DFS, whereas in patients with grade 2–3 tumors, HR negative, and ductal histology, the achievement of a pCR was associated with a better outcome. When considering the different breast cancer subgroups, no impact of pCR on prognosis was seen in luminal A-like and in luminal B/HER2-positive tumors, whereas a great impact was seen for HER2-positive, TNBC, and luminal B/HER2-negative tumors [41]. In the CTNeoBC meta-analysis, a total of 12 trials including 11,955 patients and including almost all studies of the German meta-analysis were analyzed. The study confirmed that pCR (ypT0 ypN0) was associated with a better outcome with a greater effect observed in aggressive subtypes (TNBC, HER2 positive HR negative) [63]. The magnitude of pCR improvement needed to predict long-term clinical benefit is still a matter of debate. Based on the results of the CTNeoBC trial, the FDA recognized ypT0/is ypN0 as the preferred definition for pCR as a regulatory end point, mainly because the use of the more stringent definition could result in a lower rate of pCR, affecting the real benefit of the drug. In contrast to the previous data, a meta-regression analysis of 29 studies based on data extracted from the literature failed to support the use of pCR as surrogate end point for DFS and OS. However, an increased level of surrogacy of pCR was found in trials comparing intensified/dose-dense regimens vs. standard regimens [17]. Even if an association between pCR and outcome has not yet been proven for all patients affected by breast cancer, several studies support this association for HER2-positive tumors [30, 63]. Further doubts about the value of pCR as a surrogate end point have been underlined by the results of the ALTTO trial that investigated the use of trastuzumab and/or lapatinib in the adjuvant setting. In contrast to the NeoALTTO trial, the ALTTO study did show only a marginal but nonsignificant reduction in disease recurrence after a median follow-up of 4.5 years [64]. This result goes in line with the other neoadjuvant trials that did not show a significant improvement of pCR by lapatinib [35, 38]. Moreover, the analysis of Korn and colleagues fails to provide sufficient evidence to the use of pCR to recommend a treatment based solely on a positive trial result or eliminate a new agent from further development based on a negative trial results [65].

Recommendations

1. pCR surrogacy has still to be proven. Given the available data, every effort should be taken to achieve a pCR at least in aggressive phenotypes, especially in HER2-positive tumors.

43.7 Residual Disease After Neoadjuvant Chemotherapy

In order to define the prognosis of patients with residual disease after NACT, several classifications could be applied. The German Breast Group previously described the correlation between outcome and the TNM staging system [41]

that remains a valid tool also in the post-NACT setting. Currently, other widely used classification systems are the clinicopathological stage and biological markers (CPS-EG) score, the residual cancer burden (RCB) score, and the integrated score of RCB and Ki67 (RPCB) score. The integration of estrogen receptor status and tumor grading in the CPS score allowed the identification of seven distinct patients groups having different metastasis-free survival and disease-specific survival. In particular, HR-negative disease and G3 tumors were additional independent risk factors [22, 66, 67]. The RCB score is calculated as a continuous index combining pathological measurements of primary tumor and nodal metastases to predict distant relapse-free survival. In particular the presence of an extensive residual disease is associated with poor prognosis, irrespective of the type of neoadjuvant chemotherapy administered, adjuvant hormone therapy, or the pathological stage of the residual disease [68]. Given that high posttreatment Ki67 values measured on the surgical excision specimen are independently associated with poorer DFS and OS, the integration of posttreatment Ki67 with RCB provides more prognostic information than the two variables alone [69].

No agent is currently approved for patients with residual disease after NACT. The NaTaN study failed to demonstrate an improvement in terms of DFS in patients treated with zoledronate as post-neoadjuvant therapy after standard anthracycline-taxane-based NACT [70]. In contrast the CREATE-X trial demonstrates a 2-year DFS benefit of adding capecitabine to standard adjuvant treatment in patients with HER2-negative residual disease after anthracycline-taxane-containing NACT (2-year DFS with/without capecitabine, 87.3% vs. 80.5%; $p = 0.001$) [25]. Given the high risk of these patients and the lack of data deriving from the post-neoadjuvant setting, they should be referred for participation in clinical trials. Several studies are ongoing to define if new agents could improve the prognosis of patients with residual disease after a preoperative chemotherapy. The PenelopeB study (NCT01864746) investigates the role of palbociclib in addition to standard endocrine therapy in patients with a CPS-EG score ≥3 or 2 but with metastatic lymph nodes after NACT. The Katherine study (NCT01772472) compares the use of trastuzumab vs. TDM-1 as adjuvant therapy in patients with HER2-positive breast cancer who have residual tumor in the breast, whereas the Olympia trial (NCT02032823) investigates the role of olaparib as adjuvant therapy in TNBC patients harboring germline *BRCA*1/2 mutations.

Recommendations

1. Patients with HER2-negative residual disease after anthracycline-taxane NACT could derive further benefit from the addition of capecitabine as part of standard adjuvant chemotherapy [25]. Given the few data available on post-neoadjuvant chemotherapy for patients who failed to achieve a pCR, the participation in clinical trial is still a recommended option.
2. Patients with HR-positive disease should start endocrine treatment. Patients becoming HR positive after NACT should also be considered for antihormonal therapy.
3. Patients with HER2-positive disease should complete trastuzumab therapy for up to 1 year.

Conclusion

The neoadjuvant setting provides the unique opportunity to directly observe the effect of treatment on the tumor and to apply new strategies to nonresponding patients to increase their outcome. Further research is needed in order to better select the right patients for the right therapy in order to maximize the benefit and minimize toxicities.

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Treatment of Advanced Disease: Guidelines

44

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44.1 Introduction

Breast cancer (BC) is the most prevalent malignancy in women and the second leading cause of cancer-related death in developed countries. In 2015, in the United States, about 231,840 new cases of invasive BC will be diagnosed in women, and 40,290 women will die from this disease [1]. In Europe, there were an estimated 464,000 new BC cases and 131,000 BC-related deaths in 2012 [2]. The majority of breast cancer-related deaths are associated with metastatic disease. Over the past decades, we have observed a stage migration, with a greater proportion of patients being diagnosed with early breast cancer. Nonetheless, 6–10% of women diagnosed with BC present with metastatic disease ab initio in developed countries. The percentage reaches 50–60% in developing countries [3]. Additionally, 20–50% of patients first diagnosed with early BC will eventually develop metastatic disease [4].

Nomenclature wise, the words advanced, metastatic, and secondary are all used to define stage IV breast cancer. Following the ABC guidelines [5], we will consider in this chapter that advanced breast cancer (ABC) includes metastatic and inoperable locally advanced disease. For consistency, we will mostly use advanced breast cancer (ABC) throughout the text.

Significant advances in the treatment of patients with ABC have been observed in the last 30 years. More therapeutic options are available nowadays, contributing to an improvement in the overall outcome of this disease and in the quality of life of patients with ABC. Nevertheless, very few of these treatments have provided a survival benefit, and the prognosis remains poor with a median overall survival (OS) of only 2–3 years [6, 7]. ABC continues to be generally incurable, but it is a treatable disease, and some patients can live with it for extended periods of time. The main treatment goals in this setting are to control symptoms and to extend survival, without compromising quality of life. These treatment goals should be discussed with the patient, in an accessible and comprehensive way, always respecting individual characteristics, beliefs, and cultural differences. Although less common, a very important subset of patients with ABC, for example, those with oligometastatic disease (defined as low-volume metastatic disease with limited number and size of metastatic lesions—up to 5 and not necessarily in the same organ [8]—or low-volume metastatic disease that is highly sensitive to systemic therapy), can achieve complete remission and a long survival. A multimodal approach, including local-regional treatments with curative intent, should be considered for these selected patients.

BC is a heterogenous goup of diseases which results from various molecular alterations; activation/inhibition of different cellular pathways separates it into different subtypes, characterized by different clinical behavior and response to various treatment options. As more information regarding the biology of BC has emerged, various tailored treatments are currently available according to specific BC subtype, resulting in improved outcomes, specifically in the HER-2-positive ABC and, more recently, in luminal ABC.

According to the German Tumor Registry Breast Cancer study [9] that included 1409 ABC patients, the proportional distribution of the BC subtypes in the advanced setting does not differ much from the distribution in the early setting. These researchers have found that 58% of cases were hormonal receptor (HR) positive/HER-2 negative, 19% were triple positive, 13% had triple-negative disease, and 10% of the patients had HR-negative/HER-2-positive breast cancer. This study also confirmed that ABC patients usually receive a substantial number of lines of therapy, with 60% of HR-positive population and 40% of the HR negative receiving at least three lines of treatment.

The management of ABC not only depends on various tumor-related factors but also on patient characteristics and previous drug exposure. As a general idea, patient's age should not be the only factor when deciding to withhold

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treatment (in elderly patients) or to overtreat (in young patients) [10–12]. Taking into account the complexity of the disease and also the management, ABC patients should be treated in a specialized breast unit, where all appropriate specialties (including, but not restricted to, medical, radiation, surgical oncologists, imaging experts, pathologists, gynecologists, nurses, psycho-oncologists) forming a multidisciplinary team could be involved. Although routinely applied in the early breast cancer setting, a multidisciplinary expert approach is not always offered to ABC patients. The development of the international advanced breast cancer (ABC) consensus guidelines has reinforced this need and contributed to the development of international standards and to the improvement in advanced breast cancer care.

There are few proven standards of care with high level of evidence in ABC management. Clinical trials addressing important unanswered clinical questions in this setting are urgently needed. Whenever clinical trials are available, patients should be offered inclusion in well-designed, prospective, independent trials [10].

44.2 Diagnosis and Staging Recommendations

The staging evaluation of women who present with metastatic or recurrent breast cancer should always include a history and physical examination, complete hematology, and biochemistry tests including liver function tests, renal function, electrolytes, calcium, total proteins and albumin, and imaging of the chest, abdomen, and bone [13]. Although patients with HER-2-positive or triple-negative MBC have a higher probability of CNS metastatic disease, current recommendations do not support routine brain imaging in asymptomatic patients [5]. Positron emission tomography scan (PET scan) should not be routinely part of the staging workup but should be used selectively, namely, following equivocal findings on conventional imaging techniques when a relapse is suspected [14] or to confirm the diagnosis of oligometastatic disease. The usefulness of serum tumor markers in BC is not well established for diagnosis or follow-up after adjuvant therapy. However, if initially elevated, tumor markers such as CA15-3, CEA, or CA-27.29 may aid in evaluating response to therapy, particularly in patients with nonmeasurable metastatic disease [5]. Tumor markers should not be used alone for treatment change decisions, in particular in the beginning of treatment. An early rise in tumor marker levels during the first 4–6 weeks of a new therapy can be a result of a tumor flare [15].

Biopsy of metastatic disease at presentation or at first recurrence of disease should be performed, if easily accessible, in order to confirm the diagnosis of metastatic disease and to test for hormone receptors (estrogen receptor and progesterone receptor) and HER-2 expression [5, 16]. Depending on the metastatic site (e.g., bone tissue), technical considerations need to be discussed with the pathologist. Adequate characterization of the BC phenotype will allow better definition and selection of treatment strategy. Several reports have demonstrated discordance between ER/PR and HER-2 status of primary tumor and corresponding metastases [17–19]. The reasons for this discordance may be related to clonal selection during tumor progression, intratumoral heterogeneity, selective pressure from therapy, independent evolution of a clone in both sites, or false shifts related to evaluation including tissue processing and scoring interpretation [17]. If the results of tumor biology in the metastatic lesion differ from the primary tumor, it is currently unknown which result should be used for treatment decision making. Following ABC recommendations, targeted therapy (ET and/or anti-HER-2 therapy) should be considered when receptors are positive in at least one biopsy, regardless of timing [5].

44.3 Treatment Recommendations

BC is a heterogeneous disease with diverse clinicopathological features, deregulation of distinct signaling pathways, and different drug sensitivity. Selecting therapies in ABC must therefore take into account both the biology and disease extent and patient characteristics. Many factors must be considered for tailoring the decision in this setting, always giving priority to the patient's preferences; these factors are described in Table 44.1.

Table 44.1 Factors to consider in treatment decision making for ABC patients

Disease-related factors	Hormonal receptor status HER-2 status BRCA 1/2 mutation Disease-free interval Tumor burden (defined as number and site of metastases) Need for a rapid disease/symptom control
Patient-related factors	Patient's choice Biological age Performance status Comorbidities Liver, renal, heart function Menopausal status Socioeconomic and psychological factors
Treatment-related factors	Previous therapies received Response to previous therapies Toxicity of previous therapies Availability of therapies

Note: Adapted from ABC 2 [10]

44.3.1 Hormone Receptor-Positive/HER-2-Negative Advanced Breast Cancer

HR-positive BC is the most common subtype among ABC patients, representing approximately 75% of cases [20]. In these patients, endocrine therapy is an effective treatment [21] and should be used as first option, even in the presence of visceral metastasis, unless there is immediate life-threatening disease or visceral crisis, in which case chemotherapy should be considered [22]. According to the ABC 2 consensus guidelines, "visceral crisis" is defined as "severe organ dysfunction as assessed by signs and symptoms, laboratory studies and rapid progression of disease, implying important visceral compromise, not only the presence of visceral disease" [8]. The use of endocrine therapy in HR-positive ABC is supported by its mechanism of action, low toxicity profile, and lower response of this type of tumors to chemotherapy [20].

All international guidelines [8, 10, 16, 23] recommend ovarian suppression/ablation combined with additional endocrine therapy as the first choice for premenopausal women. The additional endocrine agent can be an aromatase inhibitor or tamoxifen, according to the type and duration of prior adjuvant endocrine therapy. Fulvestrant is also a valuable option, but fewer data exists regarding its use in premenopausal patients, and for the moment it requires concomitant ovarian suppression/ablation [5, 10].

For postmenopausal women, the preferred first-line endocrine therapy depends on the type and duration of adjuvant endocrine therapy, as well as the time elapsed from the end of adjuvant endocrine therapy. An aromatase inhibitor, tamoxifen, and fulvestrant are all acceptable options [5, 10, 16].

The addition of everolimus to an aromatase inhibitor is an option for some postmenopausal patients with disease progression after a nonsteroidal aromatase inhibitor, since it significantly prolongs PFS albeit not OS [5, 24]. Due to its toxicity profile and lack of overall survival benefit, the decision must be taken on a case-by-case basis, after careful discussion with the patient and with adequate education of a preventive measure for the most common toxicities.

In the phase 2 trial PALOMA-1/TRIO-18 [25], the addition of palbociclib (a CDK4/6 inhibitor) to an aromatase inhibitor yielded a substantial PFS benefit, as first-line therapy for postmenopausal women. However, these results must be confirmed in the phase 3 trial, PALOMA-2, which has already finalized accrual, before becoming a recommended option.

The addition of palbociclib to fulvestrant, mostly in second-line therapy for post-, peri-, and premenopausal patients, has provided a PFS benefit in the PALOMA-3 trial [26]; due to its favorable toxicity profile and improvement of quality of life, despite not yet providing OS benefit, it can be considered as a treatment option in this setting. For pre-/perimenopausal patients, the addition of an LHRH agonist is needed [27].

Concomitant chemotherapy and endocrine therapy have not shown a survival benefit and should not be performed outside a clinical trial [10].

44.3.2 Triple-Negative Breast Cancer

Triple-negative breast cancer (TNBC) identifies invasive BCs that lack the expression of ER, PR, and HER-2 and accounts for 15% of all BC [28]. Generally, patients with metastatic TNBC have a poorer prognosis compared with women with other ABC subtypes, with a median survival of only 13 months [29, 30]. There is no specific systemic regimen for sporadic (non-BRCA associated) TNBC and little data to support treatment selection [31, 32]. Platinum agents, including carboplatin and cisplatin, may be of special interest in cells that are deficient in homologous recombination repair mechanisms such as BRCA-mutated cells. Evidence from preclinical and some clinical studies seem to confirm the efficacy of this strategy [33–35]. In particular, recent phase 2 randomized trials demonstrated improved pCR rates in patients treated with neoadjuvant treatment that included a platinum compound [36, 37]. In metastatic TNBC patients previously treated with anthracyclines with or without taxanes in the (neo)adjuvant setting, carboplatin demonstrated comparable efficacy and a more favorable toxicity profile, compared to docetaxel, in the TNT UK trial, and is therefore considered an important treatment option [10, 38, 39].

44.3.3 HER-2-Positive Breast Cancer

The amplification of HER-2 occurs in approximately 20% of all BC and was associated with a more aggressive disease phenotype, with a poorer prognosis and shorter overall survival [40]. The development and approval of trastuzumab, the first HER-2-targeted therapy, has changed significantly the natural history of HER-2-positive ABC patients. Since then, several other HER-2-targeted therapies have been approved for the treatment of this BC subtype. In fact, HER-2-positive ABC is probably the subtype for which highest level of evidence exists for the largest number of management strategies. Level 1 evidence supports the recommendations for early (as first line) administration of anti-HER-2 therapy to all patients with HER-2-positive ABC, except in the presence of contraindications, and for continuing anti-HER-2 therapy with subsequent treatment in patients progressing on an anti-HER-2 agent combined with chemotherapy or endocrine therapy [5, 8, 10, 16].

For patients with ER+/HER-2-positive ABC for whom endocrine therapy was chosen over chemotherapy, the use of anti-HER-2 therapy (either trastuzumab or lapatinib) in combination with endocrine therapy for highly selected patients can be considered [8]. Moreover, if chemotherapy plus anti-HER-2 therapy was chosen as first-line therapy and provided a benefit, it is reasonable to use endocrine therapy plus anti-HER-2 therapy as maintenance therapy, after stopping chemotherapy, although this strategy has not been studied in clinical trials [8].

It has become a not-so-uncommon situation to have ABC patients with HER-2-positive tumors in complete remission for long periods of time. An important clinical question is when to stop the anti-HER-2 therapy in these cases. The ABC consensus [10] suggests that stopping the anti-HER-2 therapy, after several years of sustained complete remission, may be considered in some patients, particularly if treatment rechallenge is available in case of progression [8].

The current preferred first-line therapy, for patients previously untreated with anti-HER-2 therapy, is the triplet trastuzumab + pertuzumab + chemotherapy, which has been shown to improve PFS and OS in the CLEOPATRA trial [41]. For patients previously treated in the (neo)adjuvant setting with anti-HER-2 therapy, the combination of chemotherapy + trastuzumab + pertuzumab is an important option for first-line therapy, but not the only standard of care since very few (88) of these patients were treated in the CLEOPATRA trial and all with trastuzumab-free interval >12 months. In addition, in the MARIANNE trial [42], dual blockade with T-DM1 and pertuzumab was not superior to T-DM1 alone nor to trastuzumab plus chemotherapy (taxanes) in the first-line setting.

For patients who relapse either on or within 12 months of adjuvant trastuzumab, there are currently no data regarding the best treatment strategy, since these patients were excluded from the CLEOPATRA [41] and MARIANNE trials [42]. This is therefore a research priority, in view also of their bad prognosis.

Results from the EMILIA [43] and TH3RESA [44] trials support the use of T-DM1 as the standard of care for patients with disease progression after treatment with at least one line of trastuzumab-based therapy. However, there are no data on the use of T-DM1 after dual blockade with trastuzumab and pertuzumab.

In case of progression on trastuzumab-based therapy, the combination of trastuzumab and lapatinib is a reasonable treatment option for some patients and may actually be one of the few indications of this agent in ABC, since phase 3 randomized trials [45–47] have proven that combinations of chemotherapy and trastuzumab are superior to combinations of chemotherapy and lapatinib.

In view of these new compounds recently approved in HER-2-positive ABC, optimization of sequencing and combining strategies and better predictive markers of response are of paramount importance.

44.3.4 Cytotoxic Therapy

Classic chemotherapy still plays an important role in the treatment of ABC. Unlike the adjuvant setting, in which the goal of therapy is cure, the aim of therapy in the setting of ABC is essentially palliation. Therefore, besides efficacy, tolerability and quality of life are major factors that need to be taken into account when evaluating potential gains in disease response and survival.

In recent years, the patterns of the use of chemotherapy in ABC patients have changed, and in the majority of patients, sequential single-agent therapies are preferred over aggressive multidrug regimens. Several randomized trials and a Cochrane meta-analysis provide level 1 evidence for the recommendation to preferably use sequential monotherapy, since the overall efficacy is similar to combinations and the toxicity and quality of life are better [47–56]. Therefore, all international guidelines recommend that sequential single-agent therapy should be the preferred choice for most ABC patients, except in cases of rapid progression, visceral crisis, or highly symptomatic disease [10, 16]. This strategy will allow in patients not requiring rapid tumor shrinkage, significantly lower toxicity without compromising efficacy, and disease control.

Metronomic chemotherapy is also a good treatment option for patients not requiring rapid tumor response and has a very favorable toxicity profile. The better-studied regimen is CM (low-dose oral cyclophosphamide and methotrexate), but others, such as capecitabine and oral vinorelbine, are being evaluated [57, 58].

44.3.5 Specific Sites of Metastases

In recent years, the role of local treatment of metastatic lesions in patients with ABC has been growing. Besides surgery, alternative modalities such as stereotactic radiotherapy or tumor embolization with isotope-loaded microspheres may be considered for the local treatment of metastatic lesions.

According to some retrospective data, oligometastatic disease in the liver or lung can be treated with "curative-intent" surgery, providing long-term complete remissions [59]. However, the reported outcomes were in a highly selected patient population, and, although encouraging, this approach can only be considered in selected cases with good performance status, limited liver/lung involvement, and no extrahepatic or extrapulmonary lesions and after adequate disease control by systemic treatment [10]. Moreover, further

prospective studies evaluating the impact of local treatment on survival are needed. Furthermore, a multidisciplinary team involving medical oncologists, surgeons, radiation oncologists, and radiologists is crucial to define the best therapeutic strategy for each individual patient.

In patients with bone metastasis, further radiological assessments that could indicate signs of pathological fractures are recommended in case of persistent and localized pain. In case of fracture of a long bone, an orthopedic evaluation is required in order to establish the indication for surgery, which is generally followed by radiotherapy. In the absence of a clear fracture risk, radiotherapy is recommended [10]. In cases of neurological symptoms and signs suggestive of spinal cord compression, further investigations should be urgently recommended in order to identify one or multiple concomitant lesions. Due to increased sensitivity, MRI is preferred over CT scans. If immediate decompressive surgery is not optional, emergency radiation therapy should be performed. The early use of a bone-modifying agent (bisphosphonate or denosumab) in combination with other systemic therapy is supported by different international recommendations [5, 60, 61]. In cases of oligometastatic disease, with an isolated bone lesion, it is not clear to date which is the optimal regimen and duration of the bone-modifying treatment, but there is no strong reason to stop after 2 years as it was initially recommended. Radiotherapy should be offered to patients with painful bone metastasis and for the management of spinal cord compression [62]. In cases of isolated bone lesions, stereotactic body radiotherapy or vertebroplasty should be considered, with the goal of delaying morbidity associated with the lesion and maintaining/improving quality of life.

Brain metastases are relatively frequent in patients with HER-2-positive and triple-negative ABC. In HER-2-positive ABC, brain involvement occurs later, and the outcome is dependent on the response to anti-HER-2 therapy and control of extracranial disease. In patients with triple-negative ABC, brain metastases appear earlier in the course of the disease and are associated with a poorer outcome. In recent years, the role of local management has increased for brain metastases. Neurosurgery development has been associated with a decrease in perioperative mortality, and the introduction of noninvasive techniques such as stereotactic radiosurgery has allowed for the use of less toxic approaches in selected patients. In patients with a single or a small number of brain lesions, surgery or radiosurgery should be used [63, 64]. If surgery or radiosurgery is performed, it may be followed by whole-brain radiotherapy, but this should be discussed in detail with the patient, balancing the longer duration of intracranial disease control and the risk of neurocognitive effects [10]. In fact, whole brain radiotherapy is now delayed as much as possible, especially in HER-2-positive ABC since these patients can now live several years. For other cases, where these less toxic options are not feasible, whole-brain radiotherapy is the treatment of choice [5]. In patients with HER-2-positive ABC with brain metastases and stable extracranial disease, systemic therapy should not be changed [8]. For patients with HER-2-positive cancers where brain metastases are the only site of recurrence, the addition of chemotherapy to local therapy is not known to alter the course of the disease. It is recommended to restart the anti-HER-2 therapy (trastuzumab) if this had been stopped [8].

44.3.6 Locoregional Treatment of the Primary Tumor in De Novo Stage IV Patients

Several retrospective series and a meta-analysis [65] of these retrospective data have suggested a survival benefit associated with the removal of the primary tumor in patients with de novo stage IV breast cancer. To achieve that survival benefit, the surgery must be performed with the same quality as in early breast cancer, i.e., complete removal of the tumor and management of the axilla. There is no evidence to perform the so-called palliative mastectomy except in cases of need to control local symptoms such as bleeding or ulceration and where the surgical approach could improve the quality of life. Even in those cases, palliative radiotherapy must also be discussed as an alternative option.

More recently, two small but prospective randomized studies were [66, 67] presented and did not confirm the survival benefit. These were small studies, with different timing and patient selection for surgery, and do not yet provide a definite answer to this important question.

However, it is now recommended to consider this surgery only in highly selected patients, for example, those with bone-only disease, after careful discussion with the patient [8].

Further clinical trials evaluating this approach concerning the timing, patient population, and methods are currently ongoing [8].

44.4 Follow-Up of Patients with Advanced Breast Cancer

In order to effectively manage patients with advanced/metastatic disease, serial evaluation is a key component of the care that clinical oncologists must provide. There is currently a lack of evidence from prospective randomized clinical trials comparing surveillance strategies in patients with ABC. Thus, information must be drawn from current available guidelines (ABC, AGO, ASCO, ESMO, NCCN) and from common clinical practice.

The goals for evaluation and follow-up of patients with ABC are to manage symptoms associated with the disease and treatment and to direct therapy, in an effort to maximize both length and quality of life [13]. Therefore, assessment should include review of toxicities, symptoms and quality of life evaluation, physical examination, imaging, and blood tests. Strong consideration should be given to the use of validated patient-reported outcome measures (PROMs) for patients to record the symptoms of disease and side effects of treatment experienced as a regular part of clinical care. These PROMs should be simple and user-friendly to facilitate their use in clinical practice. Systematic monitoring would facilitate communication between patients and their treatment teams by better characterizing the toxicities of all anticancer therapies. This would permit early intervention of supportive care services enhancing QoL [8]. As an important prognostic factor, performance status should be assessed at each visit, as it may have a major impact on treatment decisions and overall goals of care. In cases where patients are being treated with oral regimens, adherence to treatment should also be assessed. Initial radiological evaluation of response to treatment should be performed 2–4 months after beginning each line of treatment for endocrine treatment or after 2–4 cycles for chemotherapy, depending on the dynamics of the disease and type of treatment [13]. The timing and interval between subsequent evaluations should be adapted to the clinical situation and disease aggressiveness. Most commonly, imaging studies will include CT scans of the chest/abdomen and bone scans. Routine pelvis CT, although performed in some countries, has a very low yield, adds cost, and appears unnecessary in most cases. 18F-Fluorodeoxyglucose (FDG)-PET and PET-CT can define extent of disease and demonstrate alterations in tumor size and metabolic activity over time; however, robust data demonstrating cost-effectiveness relative to CT/bone scan-based approaches are lacking. In this context, PET-CT is not recommended for routine staging of ABC patients, but it can be used to confirm oligometastatic disease and relapse or progression in case of doubt [8]. In patients where cord compression is suspected, MRI is the modality of choice.

In patients with bone metastases, bone scans remain the mainstay of evaluation, since data from a meta-analysis has failed to demonstrate significant benefits of PET over bone scan in this context. Interpretation of bone scans must be cautious during the first months of treatment, since a possible "flare" may be observed.

If progression of disease is suspected, additional testing should be performed irrespective of the interval from the last set of staging evaluations.

Therapy for ABC should be continued as long as the therapeutic index remains positive. There is no evidence that changing to an alternative therapy (endocrine or chemotherapeutic) regimen before progression is beneficial.

Although currently not in clinical practice, detection of circulating tumor cells (CTCs) might be useful for the follow-up of ABC patients. Several studies have shown that the dynamics of CTCs after treatment initiation are a useful predictor of treatment efficacy in ABC, being associated with progression-free survival [26, 68, 69]. In the SWOG 0500 study, patients with metastatic disease and elevated CTCs under systemic treatment are randomly assigned either to continue current therapy (until evidence of disease progression, evaluated by traditional evaluation) or to make an early switch to an alternative treatment [13]. CTCs also reflect tumor biology. Their unique phenotypic characteristics and the possibility of collecting sequential blood samples may potentially allow for real-time monitoring of treatment efficacy and for better defining the adequate treatment strategy. For example, in the NCT01185509 trial, trastuzumab-based chemotherapy will be offered to patients with HER-2-negative BC according to biopsy and with HER-2-positive CTCs. In the EORTC Treat CTC trial, patients with HER-2-positive CTCs are also randomized to receive trastuzumab.

Conclusion

In the management of ABC patients, one of the most important elements is symptom control, as sooner or later all patients with metastases will have symptoms related to organ involvement, treatment, or both. This aim is frequently challenging, as the strategy involves frequent and close communication with the patient, family, and other caregivers. However, along with disease control, ensuring and maintaining quality of life for ABC patients is crucial and mandatory and must be sought.

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Endocrine Therapies in the Adjuvant and Advanced Disease Settings

45

Olivia Pagani

45.1 Introduction and Background

Hormone receptor-positive (HR+) breast cancer (BC) is the most common histological subtype across all age groups, but the proportion of HR+ BC is inversely correlated with age [1–4]. The therapeutic manipulation of endogenous estrogen levels and/or the estrogen receptor interaction is the milestone of adjuvant and palliative therapy in female patients with HR+ BC, i.e., estrogen receptor positive (ER+) and/or progesterone receptor positive (PR+). As a consequence, the accurate assessment of HR status is critical for the optimal use of endocrine therapy (ET). Over the years, HR determination has often been inaccurate and irreproducible, with variable thresholds for positivity (e.g., ≥1%, ≥10%, any) [5], significantly impacting interpretation of trial results. The 2010 joint American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of ER and PR established a cutoff of at least 1% of positive tumor cells for a specimen to be considered positive [6]. The degree of positivity provides valuable predictive and prognostic information to plan treatment strategies: several studies showed patients with higher HR levels have a higher probability of positive outcomes when treated with ET [7–11]. For the few patients reported as ER−/PR+, repeating testing on another tissue sample is recommended to rule out a false-negative or false-positive result which could influence treatment efficacy. Retesting is also recommended in case of ER- and PR-negative results in tumor subtypes (i.e., tubular, lobular, and mucinous) almost always associated with HR positivity [6]. The absence of benefit from ET for women with ER− BC has been confirmed in large overviews of randomized clinical trials.

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45.2 Early Breast Cancer

45.2.1 Indications

Precise assessment of menopausal status is important when deciding the optimal ET in the individual patient. The available biomarkers to determine the postmenopausal status [follicle-stimulating hormone (FSH), estradiol, inhibin B, and anti-Müllerian hormone (AMH)] are of limited availability, reliability, or reproducibility. Practical guidelines to properly discriminate pre- and postmenopausal patients have been developed [12]: in general, women >60 years, after bilateral oophorectomy, and <60 years not using oral contraceptives or hormone replacement therapy (HRT) with an intact uterus and amenorrhea for at least 1 year can be considered postmenopausal. On the contrary, women having regular periods without using oral contraceptives or HRT can be classified as premenopausal. The most difficult clinical situation is defining and managing the perimenopausal transition period [13]: cautious decisions and careful monitoring should be made in these patients.

45.2.2 Tamoxifen

Tamoxifen is a selective ER modulator (SERM) used for over 40 years to treat HR+ BC.

The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analyses repeatedly reported the benefits of adjuvant tamoxifen in pre- and postmenopausal women with HR+ BC regardless of age, the use of chemotherapy, and nodal status. In the 2011 overview [8], 5 years of tamoxifen compared to no ET were associated with a 15-year risk reduction of 39% in BC recurrence and of 30% in BC mortality. Efficacy is evident even at relatively low levels of ER positivity and independent of PR status: in ER+ disease, the absolute recurrence reduction at 15 years seems somewhat greater in ER+/PR poor disease than in ER+/PR+ disease,

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possibly because of the somewhat higher risk of recurrence without treatment in this tumor subtype [14]. The potential antagonism between tamoxifen and chemotherapy suggested by preclinical data [15] has not been definitively proven [16, 17]. The ongoing OPTIMA studies evaluate whether chemotherapy plus ET is better than ET alone in patients with positive nodes [18]: while waiting for new data, tamoxifen should be initiated at the end of chemotherapy, when given.

45.2.3 Premenopausal Women

The optimal adjuvant ET for premenopausal women is still a matter of debate. For over 30 years, tamoxifen for 5 years has been the gold standard in this setting. The sustained reduction in BC mortality well beyond year 10 is of particular interest in younger women. Several currently available additional therapeutic options [i.e., the combination of ovarian function suppression (OFS) to oral ET (either tamoxifen or aromatase inhibitors (AIs)] will be illustrated in the following paragraphs. The individual choice should consider the risk of recurrence, the latest scientific evidence, as well as toxicity profile, patient's comorbidities, and her personal preference.

45.3 Ovarian Function Suppression

OFS by surgical castration or ovarian irradiation was the first ET used in premenopausal patients [19]. In developed countries, this approach has been progressively replaced by gonadotropin-releasing hormone analogues (GnRHa) with comparable results [20]. Surgical castration remains a low-cost choice in developing countries. Bilateral salpingo-oophorectomy is also a valid alternative in BRCA1/2 mutation carriers who completed family planning.

The 2007 EBCTCG meta-analysis [21] looked at 11,906 HR+ premenopausal women (from 16 trials) who received GnRHa as OFS. As compared to the previous 2005 analysis [20], showing a benefit for OFS in terms of BC-related mortality and relapse rate in the absence of other systemic treatments, the updated results proved OFS to be beneficial whether used alone (recurrence risk reduction of 28%, $p = 0.08$), in addition to tamoxifen or chemotherapy (recurrence risk reduction of 13%, $p = 0.02$), or as an alternative to chemotherapy. The benefit was especially evident in young women (≤40 years of age) after adjuvant chemotherapy, either alone or in addition to tamoxifen. The latter effect is probably explained by the lack of permanent amenorrhea with chemotherapy alone in this subgroup of patients, treated with new chemotherapy regimens associated with less ovarian toxicity compared to CMF [22]. Few trials tested the addition of GnRHa to tamoxifen (±chemotherapy), and no trials compared a GnRHa against chemotherapy with tamoxifen in both arms.

The long-term results of the ZIPP trial [23] (median follow-up 12 years) were not included in the 2007 EBCTCG overview. The trial randomized 2710 patients into four arms: 476 patients (17.5%) did not receive any adjuvant ET, 469 (17.3%) received single-agent goserelin, 879 (32.4%) received tamoxifen alone, and 882 (32.5%) received the combination of goserelin + tamoxifen. ET was administered for 2 years. In all the three treatment arms, the disease-free survival (DFS) was higher than in the control arm, with no significant differences between treatments, in particular with no added benefit by the addition of GnRHa to tamoxifen. The 2009 Cochrane review [24], run in over 13,000 premenopausal women randomized in 14 trials, concluded that (1) there is no enough data to determine whether GnRHa alone is comparable to tamoxifen alone, (2) there is a trend of reduction in BC recurrence in favor of the combination of GnRHa + tamoxifen versus GnRHa alone, and (3) the association of GnRHa and chemotherapy shows no differences in recurrence or overall survival (OS) compared to GnRHa alone. The authors highlighted the need to assess the role for GnRHa when added to modern chemotherapy regimens and tamoxifen, to continue the follow-up in order to provide long-term outcomes, to compare different durations of GnRHa and the addition of aromatase inhibitors (AIs). The 2015 meta-analysis by Yan and colleagues [25] examined only trials comparing tamoxifen alone with tamoxifen plus OFS (6279 patients) and concluded that the addition of OFS to tamoxifen does not provide additional benefits in patients who did not received chemotherapy. Instead, in the subgroup with chemotherapy, the addition of OFS significantly improved OS with a mortality reduction of 24% ($p = 0.03$), possibly because these patients were considered at sufficient risk of relapse to candidate for adjuvant chemotherapy. This meta-analysis has some limitations, as stated by its authors: the results of the subgroup analyses were based on relatively small numbers of patients; the chemotherapy regimens varied across trials according to the period of enrollment as did the criteria for definition of menopausal status.

Since the abovementioned publications, the results of the International Breast Cancer Study Group (IBCSG)-led Suppression of Ovarian Function Trial (SOFT) became available [26]: 3066 premenopausal women were randomized to 5 years of tamoxifen, tamoxifen + OFS, or exemestane + OFS. Overall, at median follow-up of 5.6 years, adding OFS to tamoxifen did not provide a significant benefit in terms of DFS (84.7% in the tamoxifen group, 86.6% in the tamoxifen + OFS group; hazard ratio (HR) 0.83; 95% confidence interval [CI], 0.66–1.04; $p = 0.10$). The preplanned analysis according to the administration of chemotherapy allowed discriminating two different groups of patients and outcomes. In the low-risk patient subgroup

(mostly >40 years, with small, node-negative tumors of low-intermediate grade) who did not receive chemotherapy, >95% of patients remained free from BC at 5 years irrespective of treatment assignation. In contrast, in the cohort of patients at higher risk of relapse, who deserved chemotherapy according to the treating physician and remained premenopausal afterward, the rate of freedom from BC at 5 years was significantly higher among patients receiving tamoxifen + OFS than tamoxifen alone (82.5% and 78.0%, respectively, HR 0.78; 95% CI, 0.60–1.02). Of note, in the subset of very young patients (<35 years), BC recurred in approximately one third of the patients receiving tamoxifen alone and in one sixth of those treated with exemestane + OFS (67.7% and 83.4%, respectively), suggesting OFS plays a major role in younger premenopausal patients. SOFT data allow to better select premenopausal patients for whom tamoxifen alone is not indicated, as acknowledged in all the most recent consensus guidelines [27–30].

Data on the efficacy and safety of 3-monthly versus monthly GnRHa are scarce. In 170 Japanese women, 3-monthly goserelin was not inferior to monthly administration in terms of estradiol suppression, safety, and tolerability [31]. In clinical practice, the 3-monthly administration can be considered in older premenopausal women (>40 years): despite technical challenges, estradiol, LH, and FSH levels should be regularly checked and suppressed, as amenorrhea is not the only reliable indicator of OFS [27, 30].

45.3.1 Aromatase Inhibitors

Third-generation AIs (the nonsteroidal letrozole and anastrozole, the steroidal exemestane) efficiently block the enzyme aromatase which synthesizes estrogens from androgens and achieve a nearly complete suppression of total-body aromatization and plasma estrogen levels in postmenopausal women [32]. Conflicting evidence has questioned the benefit of AIs in overweight/obese patients: the increased body aromatization in the fat tissue may in fact induce incomplete suppression of estrogen production by AIs. In overweight (BMI ≥25 kg/m^2) premenopausal patients treated with anastrozole in the ABCSG-12 trial, the risks of recurrence and death were significantly higher (HR, 1.49; 95% CI, 0.93–2.38; $p = 0.08$ and HR, 3.03; 95% CI, 1.35–6.82; $p = 0.004$, respectively) than in patients treated with tamoxifen [33]. In the ATAC study, menopausal women with a BMI >35 had a poor prognosis compared to lean women independent of treatment (tamoxifen or anastrozole) with a nonsignificant reduced benefit for anastrozole among obese individuals [34]. In contrast, in the BIG 1-98 study, the added benefit of letrozole over tamoxifen was irrespective of BMI [35]. No correlation was found between on-treatment aromatization levels or aromatase inhibition and BMI in 64 patients treated within six different clinical trials with a panel of aromatase inhibitors [36]. While waiting for the BMI data from TEXT-SOFT in premenopausal women, there is no sound data suggesting not prescribing AIs in overweight patients, if indicated [37].

45.3.2 Postmenopausal Women

Different AI treatment algorithms have been studied: (1) head-to-head comparison versus tamoxifen for a total of 5 years, (2) following 2–3 years of tamoxifen for a total of 5 years versus AI or tamoxifen for 5 years, and (3) following 5 years of tamoxifen for a total of 10 years of ET. In comparison 1 (9885 patients from the ATAC [38] and BIG 1-98 trials) [39], the 2015 EBCTCG overview [40] showed recurrence (local-contralateral-distant) was significantly reduced (by about 30%) by AIs as compared to tamoxifen during the treatment period but not afterward, suggesting that 5 years of an AI reduces recurrence by about one third during years 5–9, as does 5 years of tamoxifen. Little follow-up data are available beyond year 10. The 10-year BC mortality is also significantly but slightly reduced (by about 15%) by AI over tamoxifen even though about half the deaths were not due to BC. In the switching comparison (12,779 patients), recurrence was significantly reduced only during the first years when the treatments differed (RR 0.74; 95% CI, 0.62–0.89; $2p = 0.002$) and not afterward (RR 0.99), if both groups received an AI: no significant further effect was evident after year 5, but little follow-up data were available beyond year 7. These smaller reductions in recurrence, as compared to the head-to-head comparison, can possibly be attributed to the shorter duration in which treatment differed. BC mortality was not significantly reduced (RR 0.89; 95% CI, 0.78–1.03; $2p = 0.11$). The highest reduction in the recurrence rate during the treatment period was observed when the switching strategy was compared to tamoxifen for 5 years (11,798 patients) (RR 0.56; 95% CI, 0.46–0.67; $p < 0.0001$) with no significant further effect afterward but lack of sufficient follow-up beyond year 10. To accommodate for different randomization criteria in the different trials, only patients who completed 2 years of tamoxifen without recurrence were included. BC mortality was not statistically reduced (RR 0.84; 95% CI, 0.72–0.96; $2p = 0.015$). The BIG 1-98 trial [39] was the only study also to explore the reverse sequencing (tamoxifen following 2–3 years of letrozole for a total of 5 years versus AI for 5 years). Letrozole followed by tamoxifen provided similar DFS and OS to letrozole monotherapy in all patient groups: despite the study was not powered to test equivalence and these results are based on few patients and events they are of interest for women who do not tolerate AI. On the contrary, letrozole monotherapy tended to be better than tamoxifen followed by letrozole, especially for

control of distant recurrence in patients at higher risk of early relapse (e.g., patients with positive axillary nodes).

No apparent differences in efficacy emerge between different aromatase inhibitors: indirect and randomized comparisons [41] show little difference between AIs.

Overall, the reduction in 10-year BC mortality with AIs compared with tamoxifen is only slight but significant. As a consequence, as stated in the last San Gallen Consensus [28], tamoxifen alone may be suitable for patients at low risk of disease recurrence, while for patients at higher risk (i.e., ≥4 positive nodes, grade 3, high proliferation index), an AI should be considered and given up front. This attitude is supported by the STEPP analysis, performed in BIG 1-98 patients, of a composite measure of prognostic risk factors (i.e., number of involved lymph nodes, grade, tumor size, presence of peritumoral vascular invasion, age, and biological characteristics) which are commonly considered in clinical practice when deciding the best adjuvant ET for the individual patient. This analysis revealed patients at lowest risk did similarly well with letrozole monotherapy, a sequence of letrozole and tamoxifen, or tamoxifen monotherapy [42].

In a retrospective analysis of the BIG 1-98 trial, the magnitude of benefit of adjuvant letrozole seems greater for patients with lobular carcinoma ($n = 324$) versus ductal carcinoma ($n = 2599$) [43]. The small number of lobular cancers in the analysis and the unclear underlying biological mechanisms require further validation before AIs can be routinely recommended in this subset of patients. In the same analysis, no difference between letrozole and tamoxifen was reported in women with ductal carcinomas and luminal A-like subtype, defined as ER and/or PR+, HER2 negative, and with Ki-67 <14%. On the contrary, women with ductal carcinomas and luminal B-like subtype (i.e., Ki-67 ≥14%) experienced a significant reduction in the hazard of a DFS event with letrozole. This observation reinforces the role of tamoxifen in patients with favorable biological characteristics.

45.3.3 Premenopausal Women

The combined analysis of SOFT and TEXT (Tamoxifen and Exemestane Trial), comparing 5 years of exemestane + OFS with tamoxifen + OFS (4690 patients), after a median follow-up of 68 months, showed an absolute 3.8% gain in the 5-year DFS in patients treated with exemestane + OFS compared to those receiving tamoxifen + OFS (91.1% versus 87.3%, HR 0.72; 95% CI, 0.60–0.85; $p < 0.001$) [44], comparable with the benefit of AIs in postmenopausal women. Overall, 57.4% of the patients did receive adjuvant chemotherapy. Timing of chemotherapy and ET initiation was different in SOFT and TEXT: in TEXT, patients received OFS at randomization concurrently with chemotherapy, at an average of 1.2 months after surgery; in SOFT, patients completed all chemotherapy before randomization and started OFS at an average of 8 months after surgery but were allowed to receive oral ET (typically tamoxifen) while waiting for menses to resume. In women who had received chemotherapy, the rate of freedom from BC at 5 years was higher with exemestane + OFS than with tamoxifen + OFS (5.5% in TEXT and 3.9% in SOFT): the shorter time before starting OFS might explain the different treatment benefits in TEXT compared with SOFT. Among patients who did not receive chemotherapy (20.7% and 8.3% node positive in TEXT and SOFT, respectively), >97% of those who received exemestane + OFS and approximately 95% of those receiving tamoxifen + OFS remained free from BC at 5 years: these data show effective combined ET alone is associated with excellent outcomes also in node-positive patients, arguing the routine administration of chemotherapy to all premenopausal patients with HR+ disease. Overall, the 5-year OS did not significantly differ between exemestane + OFS (95.9%; 95% CI, 94.9–96.7) and tamoxifen + OFS-treated patients (96.9%; 95% CI, 96.0–97.6): longer follow-up is however needed as HR+ patients can develop late relapses.

The Austrian Breast and Colorectal Cancer Study Group (ABCSG) 12 trial randomized 1803 premenopausal patients to 3 years of goserelin + tamoxifen or anastrozole [45]. After 94.4 months of median follow-up, no DFS difference between treatments was reported, but a higher risk of death for anastrozole-treated patients was observed (HR = 1.63; 95% CI, 1.05–1.45; $p = 0.03$). Overall, after disease recurrence, 61% of patients in the tamoxifen group received AIs as opposed to only 41% of patients in the anastrozole group. ABCSG-12 and SOFT-TEXT have several differences which can potentially explain the divergent results: in particular, in the Austrian trial, the statistical power was lower (half the number of events), and treatment duration was only 3 years, which is not the current standard of care for oral ET.

These data help clinicians in selecting premenopausal women with HR+ early BC who could benefit from the addition of AIs to OFS, according to their individual risk and the toxicity profile, as recommended in all the most recent consensus guidelines [27–30].

45.4 Treatment Duration

Women with HR+ tumors show no plateau for both recurrence and OS, with a low but continuous risk of relapse and death even after 10 years [46]: the annual rate for late recurrences exceeds 2% for at least 15 years, even after 5 years of tamoxifen therapy. The analysis of 111,993 patients, diagnosed between 1990 and 2003 and included in the SEER database, showed age differences in late relapses, younger age (<40 years) being associated with the higher hazard of BC-specific mortality throughout the period of 5–10 years, irrespective of nodal status [47]. Several clinicopathological parameters (e.g., nodal status and tumor size) are also associated with an increased risk

of late recurrence. Altogether, these results may help clinicians determine which patients are the best candidates for extended ET. Further research is therefore needed to detect individual biomarkers or multigene signatures for the identification of women at high risk of late recurrence, particularly in node-negative disease.

In contrast with earlier, smaller studies [48, 49], the ATLAS [50] and aTTom [51] trials show, in almost 20,000 pre- and postmenopausal women, that continuing tamoxifen to 10 years provides a further reduction in both disease recurrence and mortality. In the ATLAS trial, at median follow-up of 7.6 years, BC recurrence was reduced by 3% (RR 0.84; CI 95%, 0.76–0.94; $p = 0.002$), breast cancer mortality by 2% ($p = 0.01$), and overall mortality by 2.48% ($p = 0.01$). The protective effect extends well over the 10 years' treatment period (RR 0.90; 95% CI, 0.79–1.02 during years 5–9 and 0.70, 95% CI, 0.62–0.90 during subsequent years), regardless of age and nodal status. Premenopausal patients constituted only approximately 9% of the study population, and statistical significance was not reached in this subgroup, likely because of the much smaller number of events: nevertheless, these results provide the only available evidence of a beneficial effect of extended ET in premenopausal patients and should be discussed on an individual basis, especially for patients at high risk of recurrence. In the aTTom trial, despite HR status was not available in a consistent proportion of patients, the longer-treatment group had fewer BC recurrences (28% versus 32%; $p = 0.003$), and BC mortality was reduced (21% versus 24%; $p = 0.06$). Overall, these results can be considered practice changing, especially in case of a significant risk of recurrence.

In the NCIC-CTG MA.17/BIG 1-97 study, patients receiving 5 years of letrozole after 5 years of tamoxifen experienced overall an improved DFS, but a significant OS benefit was evident only in patients with node-positive disease [52]. The best DFS benefit (HR 0.25; 95% CI, 0.12–0.51) was reported in premenopausal women at diagnosis who became definitively postmenopausal at the time of randomization, providing a new treatment option in this subgroup of patients, if clinically indicated.

Other two smaller trials (ABCSG 6a and NSABP-B33) confirmed the efficacy of 3-5 additional years of an AI beyond 5 years of standard tamoxifen.

Based on the available evidence, both extended adjuvant ETs with an AI after 5 years of tamoxifen in menopausal women and tamoxifen for 10 years in pre- and postmenopausal women reduce the risk of cancer recurrence. Tamoxifen for 2–3 years followed by an AI for additional 5 years, for a total duration of up to 7–8 years of therapy, is also a valuable treatment option [53]. It is not known which strategy is preferred; tamoxifen and AIs have different adverse effects which may influence treatment decisions.

In postmenopausal women who received adjuvant AIs in the first 5 years, several trials addressed different strategies of AI extension. Patients' populations are not homogeneous across trials (e.g. upfront therapy and total duration of AI), making the results difficult to interpret and translate into clinical practice. In the recently reported studies (MA.17R, NSABP-B42, DATA), a DFS benefit was shown only in the MA.17R trial, mainly driven by reduction in the incidence of contralateral disease. No survival benefit was reported so far [54]. Consequently, extended AIs should not be routinely proposed but possibly discussed in women at higher risk of relapse who did not experience significant toxicity under previous AIs.

Molecular signatures able to predict distant recurrence rates (BCI, EndoPredict, PAM50) need to be prospectively tested to define the cost-benefit ratio of extended ET.

The optimal duration of adjuvant GnRHa has not been established. In different trials, GnRHa were given for 2, 3, or 5 years, with no direct comparisons. The latest ESMO guidelines suggest at least 2 years of treatment [55]: the excellent outcome of patients treated for 3 years in the ABCSG-12 trial suggests this can be reasonable, especially in women reporting severe side effects. In the TEXT and SOFT trials, duration of both oral ET and OFS was 5 years: to date, there are no data on their extension beyond 5 years. A phase II single-arm trial evaluated, after at least 4.5 years of adjuvant tamoxifen, 2 years of OFS in combination with the AI letrozole [56]. The study was closed after only 16 patients enrolled over 3.5 years, suggesting young women may not be highly motivated to extended ET and challenging the feasibility of future studies.

45.5 Advanced Breast Cancer (ABC)

45.5.1 Indications

For patients with HR+ ABC, ET is the recommended initial treatment even in the presence of visceral metastases: chemotherapy should be reserved in case of rapidly progressive disease or proven endocrine resistance [57]. Confirmatory biopsy of metastases, where feasible, should be considered as it may confirm concordance (or discordance) of endocrine sensitivity allowing better identification of patients likely to benefit from ET [58]. Different sequential ETs can be given until disease progression, unacceptable toxicity, or development of symptomatic visceral disease. The sequential use of ETs with different mechanisms of action may prolong the duration of response, reduce the risk of resistance, and delay the need for chemotherapy [59]. Most studies addressing the combination of ET and chemotherapy showed an increased overall response rate (ORR) or an increased time to progression (TTP) but no improvement in OS with no age-related differences [60]. Trials examining concurrent versus sequential ET and chemotherapy need therefore to be conducted. The specific scenario of patients with both HR- and HER2-positive disease will be addressed in a separate chapter.

45.5.2 Available Options

The third ESO-ESMO ABC consensus conference confirmed the statement that for postmenopausal patients, the choice of first-line ET depends both on type and duration of adjuvant ET and disease-free interval (DFI) from the end of adjuvant ET. AIs, tamoxifen, or high-dose (HD) fulvestrant (i.e., 500 mg monthly) are acceptable alternatives. In the FIRST phase II study [61], HD fulvestrant proved to be superior to anastrozole in terms of OS (median OS 54.1 months versus 48.4 months; HR 0.70; 95% CI, 0.50–0.98; $p = 0.04$). These data need to be interpreted cautiously as the OS analysis was not originally planned and not all patients had OS follow-up: the preliminary results of the larger phase III FALCON trial (450 patients) showed a PFS benefit (16.6. vs 13.8 months, HR 0.797) with immature OS data. The combination of a nonsteroidal AI and LD fulvestrant (250 mg monthly) showed discordant results in two phase III trials with similar designs [62, 63]. Subset analysis in the successful SWOG study suggests a benefit in the PFS and OS for the combination therapy only in patients without prior adjuvant tamoxifen [63] to whom this strategy can be offered. In this study, the addition of fulvestrant to anastrozole significantly decreased anastrozole concentrations in a subset of patients treated with the combination, potentially affecting treatment efficacy [64].

The optimal sequence of endocrine agents after first-line ET is uncertain and depends on which drugs were used in the neoadjuvant/adjuvant and first-line ABC settings. Reasonable options include AIs, tamoxifen, fulvestrant, progestins, high-dose estrogens, and androgens [57].

For premenopausal women, ovarian suppression/ablation combined with additional ET is the treatment of choice [65]. A meta-analysis of four studies ($n = 506$) comparing GnRHa ± tamoxifen showed the outcomes were significantly improved in patients who received the combination [66]. The limited evidence available [67] and indirect comparison of data from the adjuvant setting [44] and menopausal patients [68] suggest AIs can be a valuable alternative to tamoxifen: decisions should be made according to type and duration of prior adjuvant ET, DFI, toxicity profile, and patients' preferences. Fulvestrant is also a valuable option which mandates OFS [67]. Ovarian ablation (OA) by laparoscopic bilateral oophorectomy ensures definitive estrogen suppression and contraception, avoids potential initial tumor flare with GnRHa, and represents a cost-effective alternative particularly in middle-low-income countries. Patients should be informed on the options of OFS/OA, and decision should be made on a case-by-case basis.

45.5.3 Targeting Endocrine Resistance

Several potentially targetable mechanisms of intrinsic and acquired endocrine resistance have been identified, such as ER alterations (mutations, amplifications, or translocations) and upregulation of alternative growth pathways (i.e., the HER, the PI3K/Akt/mTOR, and the CDK4/CDK6 pathways). Tumors that are both ER and HER2+ are less responsive to tamoxifen treatment [69, 70]. At central review, 7% and 10.5% of patients in the BIG 1-98 and ATAC trials overexpressed HER2, respectively: in both trials, the benefit of AIs over tamoxifen was independent of HER2 status of the primary tumor. In SOFT and TEXT, 12% of premenopausal patients had HER2+ tumors and ~60% received HER2-targeted therapy, reflecting the accrual time period. In SOFT, the addition of OFS to tamoxifen appeared to be beneficial over tamoxifen alone (HR, 0.78; 95% CI, 0.62–0.98; $p = 0.03$) [26] as previously reported by others [71]. On the other hand, in the combined TEXT-SOFT analysis, in the presence of OFS, exemestane did not confer any advantage over tamoxifen (DFS HR = 1.25; 95% CI, 0.80–1.94) [44]. HER2 central assessment and further analysis are however needed before HER2 status is used for oral ET selection in premenopausal women.

45.5.4 mTOR Inhibitors

The mTOR inhibitor everolimus has proven to be effective in postmenopausal women relapsing/progressing under AIs both in combination with exemestane in the BOLERO-2 phase III trial [72] and with tamoxifen in the phase II TAMRAD study [73]. In the BOLERO-2 trial, a significantly longer median progression-free survival (PFS) with the combination versus exemestane alone was reported (central review: 11.0 months versus 4.1 months, respectively; HR 0.38; 95% CI, 0.31–0.48; log-rank $p < 0.0001$) [74]. Several predefined or exploratory subgroup analyses [75] demonstrated the PFS benefit was irrespective of age (i.e., <65, ≥65, and ≥70 years), the administration of prior chemotherapy for ABC (6.1 months versus 2.7 months; HR 0.38; 95% CI, 0.27–0.53), and the presence of visceral disease (6.8 months versus 2.8 months; HR 0.47; 95% CI, 0.37–0.60; $p < 0.05$). In addition, everolimus increased the median PFS in patients recurring after adjuvant therapy (11.5 months versus 4.1 months; HR 0.39; 95% CI, 0.25–0.62), suggesting to be potentially effective as first-line therapy. The overall PFS advantage did not translate into a survival benefit: the median OS in patients receiving the combination was 31.0 months compared with 26.6 months in patients receiving exemestane alone (HR 0.89; 95% CI, 0.73–1.10; log-rank $p = 0.14$) [76]; one possible explanation is that the trial was not powered to detect an OS advantage as the sample size was based on the primary end point of PFS. A network meta-analysis compared the PFS of everolimus + exemestane, as reported by the BOLERO-2 trial, with that of LD/HD fulvestrant after adjuvant or first-line ET from six studies [77]. Everolimus + exemestane was more efficacious than both

LD and HD fulvestrant (HR 0.47 and 0.59, respectively). Overall, these results contrast with those of the first-line HORIZON study, wherein adding the mTOR inhibitor temsirolimus to letrozole did not improve PFS in 1112 patients with AI-naïve ABC [78]. The single-arm BOLERO-4 phase II trial, assessing the safety and effectiveness of first-line therapy with everolimus + letrozole, has completed accrual and will also provide information on the efficacy of continuing everolimus after initial disease progression (PD); patients progressing under treatment will be allowed to maintain everolimus and add exemestane until further PD or unacceptable toxicity [79]. Everolimus is being studied also in the adjuvant setting [80, 81]. The decision to give everolimus must take into account the potential relevant toxicities associated with this combination and should be made on a case-by-case basis.

45.5.5 CDK4/CDK6 Inhibitors

The randomized phase I/II PALOMA-1 study showed an impressive PFS improvement in patients treated with the combination of the CDK4/CDK6 inhibitor palbociclib and letrozole compared to letrozole alone as first-line treatment (20.2 months versus 10.2 months; HR 0.488; $p = 0.0004$). The presence of CCND1 amplification and/or p16 loss was not predictive for efficacy. No significant difference in OS has been shown so far: a preliminary analysis suggested a trend toward increased OS (37.5 months versus 33.3 months; HR 0.813; $p = 0.2105$) in the combination arm [82]. Combination therapy was very well tolerated, and common grade 3/4 toxicities seen in the palbociclib-containing arm versus the letrozole alone arm were neutropenia (54% versus 1%), leucopenia (19% versus 0%), fatigue (4% versus 1%), and anemia (6% versus 1%). On the basis of these favorable results, the FDA granted palbociclib accelerated approval as first-line treatment for postmenopausal women with HR+ and HER2− ABC pending confirmatory results from the phase III PALOMA-2 trial (NCT01740427). The double-blind phase III PALOMA-3 trial evaluated the efficacy of palbociclib + HD fulvestrant versus HD fulvestrant alone in pre- and postmenopausal women with HR+/HER2− ABC who had relapsed/progressed on prior ET [83]. Pre and perimenopausal women received also the GnRHa goserelin. At the first interim analysis, the primary end point was reached; the median PFS was 9.2 months in the combination arm and 3.8 months in the fulvestrant arm (HR 0.422; 95% CI, 0.318–0.560; $p < 0.000001$). Of note, the relative difference in PFS was independent of menopausal status, providing a new treatment option also for young patients with HR+ ABC. At the time of the interim analysis, data on OS were immature, with a total of only 28 deaths. Several trials evaluating palbociclib plus ET are in progress in the adjuvant and neoadjuvant settings, as well as in combination with chemotherapy and HER2-targeted agents. A significant PFS improvement was also reported with Ribociclib, another selective CDK4/6 inhibitor, in combination with letrozole as first-line treatment in menopausal women, myelosuppression being the only relevant associated toxicity of the compound [84]. A third agent (LY2835219, abemaciclib) is under evaluation in different disease settings.

45.5.6 Other Compounds

The encouraging results in terms of efficacy and tolerability of a small phase II placebo-controlled trial ($n = 43$) of anastrozole combined with gefitinib, an orally active EGFR tyrosine kinase inhibitor, compared to anastrozole alone [85] were not replicated in a larger phase II study ($n = 71$) with similar design [86]. Overall, both the RR, not clearly superior to ET alone, and the toxicity profile do not support further evaluation of this combination. Efficacy of VEGF inhibitors has been disappointing to date: the pan-VEGF inhibitor pazopanib is being evaluated as an add-on therapy in a phase II trial of patients with HR+, locally advanced or metastatic BC progressing on nonsteroidal AIs in the adjuvant or metastatic setting (NCT01466972). Several additional targeted agents are under evaluation in combination with ET, e.g., PI3K, SRC, FGFR, and histone deacetylase inhibitors [87].

It is currently unknown how the different combinations of ET + biological agents compare with each other and with single-agent chemotherapy and whether a targeted agent should only be combined with ET to restore endocrine sensitivity or whether it may also prevent or delay the development of resistance [88]. Appropriate patient selection based on prior treatment history and disease characteristics will become increasingly important in maximizing the potential incremental benefit from these new agents combined with standard ET.

45.5.7 Side Effects and Adherence

ET is associated with potential physical and psychosocial long-term and late effects, specific of the drugs used and their duration. Accurate evaluation of potential contraindications to specific compounds and strategies to manage the most common toxicities [29, 54, 89, 90] should be part of routine clinical care.

ET adherence and persistence are relevant and may affect disease outcomes [91, 92]. A systematic review of 29 studies in the adjuvant setting showed that at the end of 5 years of treatment, adherence ranged from 41% to 72% (59% nonadherence for tamoxifen and 50% for AIs) and nonpersistence from 31% to 73%. Age (older or younger), increasing out-of-pocket costs, follow-up care with a general practitioner instead of an oncologist, and treatment side effects were all

negatively associated with adherence and/or persistence [93]. Health professionals should routinely assess and encourage adherence to ET [54, 94] and specifically address side effects to reduce symptom burden and potentially improve adherence [90].

The most commonly reported side effects of tamoxifen mimic menopausal symptoms including hot flashes, weight gain, sleep disturbance, sexual dysfunction, and gynecologic complications which may negatively impact QoL: rare but serious toxicities include increased risks of endometrial cancer and thromboembolism. In premenopausal women, there is little uterine cancer risk or excess risk of fatal pulmonary embolism [8]. The incidence of endometrial cancer and thromboembolism is very low even with longer therapy duration (3.1% versus 1.6% endometrial cancers for tamoxifen-treated versus placebo-treated women and relative risk of pulmonary embolism of 1.87 in the ATLAS trial) [51]. As opposed to menopausal women, tamoxifen may decrease bone mineral density (BMD) in premenopausal women, although the exact mechanism remains unclear [95].

Bothersome toxicities of AIs include musculoskeletal symptoms (i.e., arthralgias, myalgias, tendonitis, and carpal tunnel syndrome), menopausal symptoms, decreased BMD and consequent increased risk of fracture, and dyslipidemia [40]. Interestingly, although all AIs have the same mechanism of action and side effect profile, some patients who are treated with more than one of the individual AIs experience a different constellation of side effects from the different drugs. A meta-analysis of seven randomized controlled trials that compared AIs and tamoxifen as adjuvant ET in postmenopausal women (30,023 patients) showed AIs were associated with increased cardiovascular disease (OR = 1.26; 95% CI = 1.10–1.43; $p < 0.001$) and bone fractures (OR = 1.47; 95% CI = 1.34–1.61; $p < 0.001$) but a decreased odds of venous thrombosis (OR = 0.55; 95% CI = 0.46–0.64; $p < 0.001$) and endometrial cancer (OR = 0.34; 95% CI = 0.22–0.53; $p < 0.001$) [96]. Switching from one class of drug to the other can be a valuable strategy for balancing serious adverse events of individual drugs. ET may also adversely affect cognition [97]: objective but not subjective cognitive function improved approximately 1 year after cessation of either adjuvant letrozole, tamoxifen, or their sequence in a subset of patients treated within the BIG 1-89 study [98].

The addition of OFS to oral ET is associated with greater menopausal symptoms, anxiety, and depression [27]: in women who develop severe side effects, the risk-benefit ratio should be discussed according to the individual risk of relapse and OFS interruption proposed. Side effects and quality of life (QoL) have been extensively analyzed in SOFT and TEXT. Overall, 16.1% of the patients in the exemestane + OFS group and 11.2% of those in the tamoxifen + OFS group completely stopped ET. Global QoL and symptom indicators were assessed every 6 months for 24 months and then every year between years 3 and 6 in 4096 patients of both trials. Patients under tamoxifen + OFS reported more hot flushes, vaginal discharge, and sweats than those under exemestane + OFS, whereas patients who received exemestane + OFS had more bone/joint pain, vaginal dryness, and greater loss of sexual interest compared with patients on tamoxifen + OFS. Nonetheless, during the treatment period, changes in global QoL from baseline were similar between the two treatment groups [99].

Genetic polymorphisms may classify low or extensive drug metabolizers of either tamoxifen, via CYP2D6, or AIS, via CYP19A1. Many attempts have been undertaken to explore the impact of ET metabolism on toxicity and outcome with discordant results, preventing the utilization of pharmacogenomic data to select the best oral ET in the individual patient [100–102].

As ET side effects are related to suppression of estrogen production or ER blockade, it has been questioned whether the development of side effects is related to ET benefit. A number of unplanned retrospective analyses evaluated the association between symptoms of ET in general, rather than specifically for tamoxifen or AIs, and BC outcome. Most but not all analyses identified a positive association between musculoskeletal toxicity and improved DFS and OS. A subset also identified associations between vasomotor symptoms and improved outcomes. Major limitations of these data include: physician-graded adverse events instead of patient-reported outcomes, with the related underreporting of symptoms and no consistent definition for musculoskeletal symptoms across studies; exclusion of symptomatic patients at baseline, not capturing baseline symptoms and global severity, which makes it difficult to interpret these findings and to possibly apply this information to drive treatment decisions in individual patients [89].

45.6 Fertility Considerations and Pregnancy

Fertility and safety of pregnancy after the disease are major concerns for many young women with early BC [103, 104]. Fertility preservation should be addressed early after diagnosis according to all the most recent guidelines [30, 105, 106]: ideally patients should be referred to a fertility specialist before starting therapy to discuss all the available options [107]. Pregnancy following BC does not seem to negatively influence DFS or OS in HR+ premenopausal patients [108, 109]. A global IBCSG-led trial (POSITIVE-IBCSG 48-14 NCT02308085) is assessing patients' safety and pregnancy outcomes of interrupting ET after at least 18 months but no longer than 30 months to attempt conception.

45.7 Future Directions and Conclusions

The current therapeutic armamentarium in early BC requires a careful evaluation of both tumor's and patient's characteristics to select the optimal class of drugs, their sequence, and duration, carefully monitoring side effects and adherence. Patient's preference, requiring adequate and complete information, is therefore a key point to ensure the excellent outcomes reported by clinical trials translate in the overall population.

Cross talks between ER and growth factor pathways and discovery of new molecular aberrations in breast tumors will allow to develop new strategies for the cure of HR+ BC, moving from the advanced disease setting to earlier disease stages. Future treatments will likely include combination of several targeted therapies with cumulative side effects and extra personal and social costs which need to be anticipated and managed. A marker-driven selection of targeted agents for each patient and the reproducible and biologically significant detection of key molecular alterations responsible for both intrinsic and acquired resistance are therefore mandatory if we want to move to precision medicine and optimal resource allocation. As a consequence, sensitive, early, and reproducible predictors and markers of response/resistance are urgently needed in ABC to avoid unnecessary and toxic therapies. This is particularly relevant as improvements in PFS not always translate into OS benefit. Different disease end points, e.g., the post-progression survival (SPP) and/or composite end points including measurements of efficacy and toxicity and patients reported outcomes, such as the ESMO Magnitude of Clinical Benefit Scale [110], need therefore to be systematically implemented and tested.

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Chemotherapy Regimens in the Adjuvant and Advanced Disease Settings

46

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Abbreviations

AC	Doxorubicin plus cyclophosphamide, 4 cycles given 3 weekly (2 weekly for dose-dense) (×4, q21 or q14)
CMF	Cyclophosphamide, methotrexate, fluorouracil, ×6 q28 (d1+d8)
FEC	Fluorouracil, epirubicin, cyclophosphamide, ×6 q21
EC	Epirubicin cyclophosphamide
(F)EC-D	Epirubicin plus cyclophosphamide (with or without fluorouracil) ×3 followed by docetaxel ×3, q21 (or q14 for dose-dense)
TC	Docetaxel cyclophosphamide ×4 q21
TCH	Docetaxel cyclophosphamide trastuzumab ×4 q21, trastuzumab continued for total 1 year
TCarboH	Docetaxel carboplatin trastuzumab ×6 q21, trastuzumab continued for total 1 year

46.1 Adjuvant Chemotherapy Regimens

The administration of chemotherapy after primary breast cancer surgery revolutionised early breast cancer management. The groundbreaking trials by Bonadonna established CMF as a benchmark adjuvant regimen that led to reduced recurrence rates and long-term survival in all postoperative EBC patients, both node-positive and node-negative [1]. Subsequently, anthracycline regimens were found to be at least equivalent or superior to CMF, followed by third-generation taxane-containing regimes that proved superior again [2]. Chemotherapy is of clear benefit in node-positive disease but also has evidence of reducing recurrence and mortality in node-negative disease.

The emergence of the importance of HER2 status and molecular subtypes changed the paradigm for treatment. It became recognised that cancers with the same clinicopathological features (size, grade, nodal status) can have vastly different prognoses and responses to treatment, depending on subtype. This in turn impacts on the decision to give chemotherapy and what sort to give. The principles for deciding whether or not to use chemotherapy are covered in another chapter; here we focus on the choice of chemotherapy regimen.

Many adjuvant regimens exist with proven efficacy, yet there is no single combination that is considered best, due to the multitude of concurrent yet uncoordinated adjuvant trials using various combinations of agents. Chemotherapy choices may be broken down into first (CMF)-, second (anthracycline)- and third (taxane combinations with or without anthracycline)-generation regimens; however, within generations, few head-to-head trials have been performed to demonstrate an optimal regimen. Increasing dose and intensity beyond certain limits was proven counterproductive in the trials of very high-dose regimens that required autologous stem cell support, which resulted in worse survival and greater long-term morbidity, and is not recommended today [3].

Selecting an optimal regimen for an individual patient requires consideration of a number of prognostic factors, possible predictive factors and careful discussion of relative risks and benefits with the patient to allow an informed decision, in much the same way as the decision whether or not to give chemotherapy in the first place. Typical factors include age and comorbidities, tumour size, grade and extent of neurovascular invasion, nodal status and hormone receptor and HER2 status. Whether intrinsic molecular subtype, surrogate definitions of subtype or prognostic genomic panels can help in this regard is the subject of debate and remains under investigation.

While the arrival of anthracycline- and taxane-anthracycline-based regimens has improved disease-free and overall survival [2], these newer combinations come at the

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price of increased toxicity, which must be weighed against the expected absolute benefit of the chemotherapy regimen. This is particularly relevant in the better prognosis cancers, e.g. luminal A with no nodal metastases. Here, the absolute survival benefit from adjuvant chemotherapy may be in the order of <5%, and, consequently, absolute differences between newer and older regimens in terms of overall survival may be negligible. Given that the long-term risk of leukaemia and congestive heart failure following anthracycline therapy is 1%, it would seem reasonable to avoid anthracycline-containing regimens in these scenarios.

Taxane-only regimens have also been compared to anthracycline combinations in an effort to find equivalent regimens that are better tolerated and reduce the risk of long-term anthracycline-related toxicity. AC has been a standard short course treatment after proving equivalent or superior to CMF in node-positive and node-negative populations [4–5]. Subsequently, a single study demonstrated superiority of TC compared to AC in node-negative and node-positive patients, suggesting a general superiority of taxanes [6]. However in another study comparing AC to single-agent paclitaxel in women with 0–3 positive nodes, AC proved to have the higher 5-year overall survival at 95% compared to 94%, despite a treatment-related death rate of 0.5% (mainly AML) vs 0% [7]. Thus, while weekly paclitaxel is a simpler, more tolerable regimen, it is not considered equivalent to polychemotherapy. Nevertheless, the degree of inferiority may be acceptable to some patients, and it remains a reasonable regimen for intermediate-risk patients unfit for stronger combinations.

Results from two trials suggest that the fluorouracil component of FEC chemotherapy does not improve survival but does contribute to toxicity. In an Italian study using a 2 × 2 design, investigators compared FEC-paclitaxel three-weekly and two-weekly and EC-paclitaxel three-weekly and two-weekly (dose-dense) [8] and showed that omission of fluorouracil had no impact on survival but did lead to a reduction in cytopenias and gastrointestinal adverse events, suggesting that the FEC-D regimen from the PACS-01 study [9] may not suffer from dropping the fluorouracil component. Similarly, the NSABP-B36 study compared six doses of FEC100 to four doses of AC, each given at 3-week intervals, and found them to be equivalent in terms of outcome, but with greater toxicity in the FEC arm [10]. It would seem reasonable then to prefer non-fluorouracil anthracycline combinations, and our practice is to use EC-D or EC-weekly paclitaxel.

46.1.1 Dose-Dense Regimens

Regimens that reduce the time interval between doses are associated with improved relapse-free and overall survival in meta-analysis [11], although the survival benefit was not entirely seen in hormone receptor-positive patients, being statistically non-significant. Dose-dense administration comes with the advantage of reducing the total length of time a patient is undergoing treatment but may be associated with increased rates of some toxicities. Granulocyte colony-stimulating factor support is mandatory to reduce the rate of myelosuppression compared to standard dosing, which adds to cost. As the improved outcome is only incremental, the use of a dose-dense regimen may be warranted mainly in the highest-risk categories where chemotherapy is expected to offer reasonable absolute benefit—triple negative or luminal B with other high-risk features. As always, patient wishes are crucial—some may be keen to try a tougher regimen in order to complete it sooner. Having commenced a dose-dense regimen, it is entirely reasonable to switch to standard dosing if it is proving intolerable.

A widely used dose-dense regimen, particularly in North America, is AC-paclitaxel, given two-weekly. This is based on the results of the CALGB 9741 trial, which demonstrated superiority of the schedule over three-weekly AC-paclitaxel [12]. Similarly, FEC-paclitaxel and EC-paclitaxel given at two-week dose intervals resulted in statistically significant improvement in relapse-free and overall survival after 7-year follow-up, compared to standard three-week dosing [8]. Of note is the fact that three-weekly dosing of paclitaxel is now recognised as inferior to the weekly dosing schedule [13], making the comparator arm in both trials a non-standard of care and limiting conclusions. Nevertheless, when a dose-dense regimen is preferred, AC or EC, followed by either two-weekly paclitaxel ×4 or weekly paclitaxel ×12, would be appropriate.

46.1.2 Bevacizumab

The use of bevacizumab in early breast cancer has been controversial and is not currently recommended in adjuvant treatment. Neoadjuvant bevacizumab has been shown to improve pCR rates, but has not led to improved long-term outcomes, although recent data from the NSABP B40 study suggests otherwise [14]. In this study of 1186 women with HER2-negative EBC, after a median follow-up for·7 years, the addition of bevacizumab led to significantly increased overall survival (hazard ratio 0·65 [95% CI 0·49–0·88]; $p = 0·004$) and non-significantly increased disease-free survival (HR 0·80 [0·63–1·01]; $p = 0·06$) [14]. This study employed a 3 × 2 factorial design to compare additional chemotherapy agents on top of the neoadjuvant AC-docetaxel backbone, which may complicate conclusions. Further studies are required, but the increased response rate, pCR rate and different toxicity profile associated with bevacizumab mean that its use as a neoadjuvant agent is not unreasonable, preferably in a trial setting.

46.2 Recommendations

46.2.1 ER-Positive, HER2-Negative EBC

In the luminal cancers, effective adjuvant endocrine therapy is considered to be of primary importance, with chemotherapy contributing less to improved outcomes. Nevertheless, there may be important differences between luminal A and B that could impact on chemotherapy choice.

Longer-term follow-up of BIG 2-98 and a pooled analysis of four trials revealed that the benefit from the addition of taxanes to anthracycline-based therapy in terms of relapse-free or overall survival is limited to ER+ patients with high Ki67 (≥14%), a marker that can separate luminal A and B [15]. We have also shown preliminary evidence that clinicopathological markers of luminal A disease (ER- and PR-positive, HER2-negative, Ki67 low) may be sufficient to identify a group of patients where the benefit of anthracycline regimens over CMF, and of taxane regimens over anthracyclines, is small or negligible [16]. This may in part be by virtue of the fact that this is a subgroup with better prognosis, and thus a significant benefit is harder to discern. There is also evidence that luminal A disease is inherently less chemosensitive, which may also lead to irrelevant differences between newer and older regimens [17]. But whether predictive or only prognostic, consideration of tumour biology is relevant.

Thus, when it has been decided that adjuvant chemotherapy is warranted, in luminal A disease, the choice of regimen may be made with greater emphasis on acceptable toxicity and dosing schedule. The shorter course TC regimen would be acceptable or even classical CMF to avoid the risk of taxane neurotoxicity, both of which avoid risk of long-term anthracycline complications. CMF however is a long six-month course CMF therefore is to be considered mainly when alopecia is an issue for the patient.

It must be noted that surrogate definitions of luminal subtype using IHC remain controversial, particularly in regard to the use of Ki67, and have not been prospectively validated. While their use cannot be formally recommended at this stage, expert review panels have condoned it in principle [18].

Bearing in mind that there is no strong evidence for selecting a single regimen over another, and that each case must be assessed on its merits, we offer the following suggestions:

46.2.2 Luminal A

For 1–3 positive nodes and large tumours (i.e. intermediate risk): TC or EC (TC preferred), CMF, weekly paclitaxel × 12 (if unfit for polychemotherapy).

For >3 positive nodes, (F)EC-D, EC-paclitaxel.

46.2.3 Luminal B

As for node-positive luminal A, except with a lower threshold for prescribing taxane-containing regimens:

Node-negative: TC (or EC, but not recommended), CMF, weekly paclitaxel (if unfit for polychemotherapy).

Node-positive: (F)EC-D or EC- paclitaxel, consider dose-dense regimen for multiple high-risk features.

46.2.4 After Genomic Assay Recurrence Risk Stratification

In the case of receiving prognostic information from gene signature models such as the 21-gene recurrence score (RS) that suggest when to use of adjuvant chemotherapy, there is still little guidance on the most appropriate regimen. Furthermore, it is unknown if one can apply other prognostic factors after RS stratification—for example, in RS high patients with luminal A-like disease (unlikely but possible), is there a degree of chemotherapy insensitivity that limits the incremental difference of taxanes over anthracyclines?

Given that a high score correlates with an aggressive phenotype and high risk of relapse, it would seem reasonable to use combined anthracycline taxane regimes in these situations. For an intermediate score, we would rely on more classical markers such as nodal status—in node-positive disease, an anthracycline-taxane-containing regimen is an option; in node-negative, if it is to be given at all, short course TC, or alternatively, if a less toxic regimen is required, weekly paclitaxel or CMF. In all cases, a discussion of the risks and benefits of newer versus older generation regimens is vital to gauge the patient's priorities and wishes.

There is now evidence that cancers with very low scores in node-negative disease have such a low likelihood of relapse that there is no benefit to be gained from adjuvant chemotherapy [19]. Ongoing trials are assessing the benefit of chemotherapy in intermediate- and high-risk scores, in both node-positive and node-negative disease. Whether this helps with chemotherapy selection is yet to be seen.

46.2.5 HER2-Positive Disease

Trastuzumab revolutionised outcomes for women with HER2-positive early breast cancer and is a vital component of their adjuvant therapy. Lapatinib, conversely, has not proven to be beneficial [20–21]. More recently developed HER2-targeting agents are under investigation in this setting—pertuzumab, an inhibitor of dimerization of HER2 and HER3, and trastuzumab emtansine (T-DM1), an antibody-drug conjugate. The addition of pertuzumab to trastuzumab neoadjuvant therapy resulted in higher pCR rates in a phase

II trial (NeoSphere) [22], and phase III trials are ongoing (Aphinity).

Recommended duration of trastuzumab treatment is 12 months, based on trials that demonstrated inferiority of six months (PHARE) [23], and non-superiority for 24 months (HERA) [24]. The main toxicity associated with trastuzumab is cardiomyopathy, which usually resolves on treatment withdrawal [25]. However, this is increased when combined with anthracyclines, even if given sequentially [26]. Trastuzumab is commenced with the adjuvant chemotherapy regimens, and taxane-anthracycline combinations are generally recommended, with trastuzumab administered with the taxane after completion of the anthracycline component.

The BCIRG006 trial investigated the potential for omitting doxorubicin from the adjuvant regimen, comparing docetaxel and carboplatin plus 52 weeks of trastuzumab (TCarboH) to doxorubicin and cyclophosphamide followed by docetaxel every 3 weeks plus 52 weeks of trastuzumab (AC-TH), as well as a non-trastuzumab arm, AC-T. They enrolled 3222 women with HER2-positive early-stage breast cancer, and results were recently reported for long-term follow-up of median 10.3 years [27]. Compared to AC-T, AC-TH and TCarboH reduced the risk of disease recurrence by 30% and 24%, respectively, and the risk of death by 36% and 24%, respectively. The difference between the trastuzumab-containing regimens was not statistically significant, although the study was not powered to compare the two directly. However, there were five times as many cases of congestive heart failure cases in the AC-TH regimen compared to TCarboH (21 cases vs. 4), a rate of 2.0% in AC-TH versus 0.4% in TCarboH and 0.8% in AC-T. Furthermore, patients treated with TCarboH had lower rates of sensory neuropathy and severe neutropenia but higher rates of anaemia and thrombocytopenia.

Unfortunately, there were no results reported for subgroups by ER status. Late relapse tends to occur more frequently in ER-positive patients compared to ER-negative and are considered a failure of endocrine therapy, while relapses due to failure of chemotherapy are usually in the first 3–5 years. Thus, with longer follow-up, a survival benefit from chemotherapy in ER-positive disease will become less apparent and may dilute or mask the benefit seen in ER-negative patients when the two groups are combined. Therefore, the AC-TH may yet be shown to be clinically and perhaps statistically superior to TCarboH in ER-negative patients.

Tumour size is an important consideration. T1a (≤5 mm) and T1b (5–10 mm) tumours were poorly represented in initial trials of adjuvant trastuzumab, making it difficult to know whether such cancers require adjuvant therapy or what combination to use. A single-arm study of tumours up to 3 cm treated with adjuvant weekly paclitaxel × 12, plus trastuzumab for 1 year, showed extremely low 3-year recurrence of 98.7%, lower still in the ER-positive or <1 cm subgroups [25]. Although this was only after median 4-year follow-up, the results are encouraging for those early-stage patients who would prefer a less toxic regimen.

For the most part, we would recommend a taxane-anthracycline regimen, in combination with 12 months of trastuzumab, in all HER2-positive disease. In the case of anthracycline contraindication, or lower-risk node-negative disease, TCarboH or other non-anthracycline-based regimens would be appropriate, such as TCH. For node-negative disease <2 cm, particularly if ER-positive and employing endocrine therapy, the weekly paclitaxel-trastuzumab regimen is suggested.

In the neoadjuvant setting, the addition of pertuzumab to trastuzumab would be reasonable, given current evidence, although it comes at significantly greater financial cost with no current evidence of survival benefit.

46.2.6 Triple Negative

Breast cancers lacking expression of both hormone receptors and HER2 are not eligible for targeted therapy, making chemotherapy the only adjuvant therapy available. Furthermore, despite being relatively chemosensitive, these cancers have a generally worse prognosis. Therefore, adjuvant chemotherapy is recommended in most cases, and the threshold for prescribing a taxane-anthracycline regimen is low. For small node-negative tumours, TC or CMF may be an acceptable, less toxic regimen.

There is considerable focus on the potential of platinum agents in cancers that have altered DNA repair mechanisms, particularly deficient homologous recombination repair mechanisms such as are seen in BRCA1-/BRCA2-mutated breast cancers [28], and high rates of pathological complete response have been seen in BRCA patients in early neoadjuvant studies [29]. However, these data were not confirmed in the GeparSixto trial, where the benefit of neoadjuvant carboplatin was seen in all triple negative patients, regardless of BRCA status [30]. At present, the use of platinum salts in early breast cancer remains investigational.

46.3 Metastatic Chemotherapy Regimens

Unlike early breast cancer in which the aim of adjuvant treatment is increased cure rate, in advanced disease, the aims of chemotherapy are increased survival, palliation of cancer-related symptoms and maintenance of quality of life. Thus, a greater importance is placed on balancing toxicity with meaningful outcomes. There is no single agent or sequence of agents that is considered optimal, allowing choices of the many chemotherapeutic options to be based on tolerability and patient-specific factors. In this vein, standard recommendations are for

the use of single-agent over combination chemotherapy, as it results in reduced adverse events with no significant negative impact on survival [31–32].

While not prolonging life, combination regimens are associated with increased response rate and progression-free survival [32–33]. Thus, they may be preferred in situations where rapid disease control is required, such as visceral crisis, multiple uncontrolled painful metastases or rapidly deteriorating performance status. Bevacizumab plus chemotherapy has similar activity with a different toxicity profile and is an alternative to doublet cytotoxic regimes. Several trials have documented significantly prolonged PFS with bevacizumab plus chemotherapy compared to chemotherapy alone, particularly in combination with paclitaxel, although none demonstrate overall survival benefit [34–35]. However, it is an expensive option, and no predictive biomarkers exist yet to help tailor its use.

Duration of treatment with each regimen is not set and is usually dictated by development of adverse effects. Current evidence suggests that continuation of treatment after achieving best response, either until progression or intolerable toxicity, is associated with longer survival [36].

In HER2-negative disease, it is recommended to start with weekly paclitaxel or doxorubicin, unless these agents were used in the adjuvant setting within the previous two years. Prior adjuvant anthracycline use per se is not an absolute contraindication to palliative use—monitoring of cardiac function can be performed before and during treatment, and while the cumulative risk of delayed cardiotoxicity must be considered, the timeframe until it appears clinically may exceed life expectancy. An alternative taxane is nab-paclitaxel, which may be attractive as it does not require steroid premedication and has similar efficacy. However, at the full 150 mg/m2 weekly dose, it is associated with increased haematological and neurological toxicity.

An alternative choice, particularly for ER-positive disease, is oral capecitabine, as it is generally very well tolerated, has reduced rates of alopecia and does not require hospital admission for intravenous administration, making it a somewhat gentler transition to cytotoxic treatment after failure of endocrine therapy. In a phase II study, capecitabine led to increased median overall survival compared to CMF, with a response rate of 30% [37].

Beyond these options, a number of other single agents have shown activity.

Vinorelbine is effective in first and subsequent line, may be given in oral form and is generally well tolerated with no alopecia [38]. Intravenous form may be less convenient for the patient but is associated with less gastrointestinal symptoms and similar efficacy [39].

Eribulin has shown significant activity in heavily pretreated advanced breast cancer—in the EMBRACE trial, eribulin improved overall survival compared to treatment of physician's choice (13.1 vs 10.6 months) [40]. In a separate trial of eribulin vs capecitabine as first-third line chemotherapy in advanced disease, there was no significant difference in survival [41], but in pooled analysis of the two trials, eribulin was associated with improved overall survival in all subgroups [42]. Neutropenia and alopecia are common, and although neuropathy may be less frequent than with taxanes, it remains a risk.

Platinum salts, gemcitabine and cyclophosphamide all have modest activity but are commonly used in combination with other agents. Platinum agents also may be of particular use in patient with evidence of homologous recombination deficiency (HRD), such as BRCA1/BRCA2 carriers. The phase III TNT trial compared single-agent docetaxel to carboplatin in women with advanced triple negative breast cancer [43], and in the BRCA-mutated subgroup, response rates were considerably higher with carboplatin than with docetaxel, at 68 and 33%, respectively. Progression-free survival was 6.8 months and 3.1 months, respectively, yet in the overall population, docetaxel had slightly greater efficacy. A genomic pattern, based on its similarity to the patterns in BRCA-mutant breast cancer, has been identified that appears to predict strongly for platinum sensitivity, even in ER-positive tumours [44]. This has the potential to become a useful biomarker for the use of platinum agents but required further validation.

46.3.1 Combination Treatments

A variety of multichemotherapy regimens have been trialled in advanced breast cancer, and the choice of regimen should be guided by careful consideration of toxicity profile, as well as prior therapies and responses. Similar combinations to those used in adjuvant treatment can be employed, although dose reduction may be appropriate to improve tolerability.

Other combinations include gemcitabine plus taxane and capecitabine plus docetaxel with overall response rates over 40%. CMF has activity and may be preferred for its low alopecia rate but has been shown to be inferior to capecitabine [45].

Capecitabine and vinorelbine have been shown to be an effective combination that is particularly well tolerated, with similar disease control rate (71%) and overall survival compared to capecitabine plus docetaxel [46–47]. The two agents have distinct mechanisms of action and, given together, appear to reduce the rate of capecitabine-related hand-foot syndrome. The combination may be preferable to patients due to the oral administration and low rates of alopecia and cytopenias.

In triple negative cancers, which can be aggressive and may have fewer opportunities to try new options, combination regimens may be more appropriate. An ongoing phase

II/III trial is assessing the combinations nab-paclitaxel plus gemcitabine, nab-paclitaxel plus carboplatin or gemcitabine plus carboplatin in first-line treatment, which may shed light on optimal doublet regimens and use of platinum agents [48].

Metronomic oral chemotherapy, where doses are administered less frequently, can be an effective way to reduce toxicity and improve compliance while still improving disease control [49–52]. Data is limited to phase II trials, but where tolerability is a major concern, this may be a preferred strategy, particularly in frail patients.

46.3.2 HER2-Positive

In HER2-postive advanced breast cancer, the general consensus is to maintain HER2-directed therapy throughout treatment, with or without concomitant chemotherapy. This consists of four agents: trastuzumab with or without pertuzumab, trastuzumab-emtansine (T-DM1) and lapatinib. All except T-DM1 may be given concomitantly with non-anthracycline chemotherapy. In ER-positive disease, once a maximal response is achieved, chemotherapy may be switched to 'maintenance' endocrine therapy while continuing HER2 blockade to improve tolerability, although there are no proven strategies for this approach. In ER-negative disease, chemotherapy should generally be continued for as long as it is tolerated and there is clinical benefit but can also be ceased once maximum clinical response is seen.

The triplet combination of trastuzumab plus pertuzumab plus taxane chemotherapy is considered standard first-line therapy in HER2-positive disease, based on the substantial overall survival benefit from the addition of pertuzumab demonstrated in the Cleopatra trial [53]. In this trial, patients receiving all three agents gained an extra 15.7 months overall survival compared to trastuzumab and docetaxel plus placebo (56.5 months versus 40.8 months, respectively), although this came at the expense of higher rates of diarrhoea, rash and febrile neutropenia.

For patients relapsing during, or within 12 months of, adjuvant trastuzumab, T-DM1 is an alternative to the Cleopatra regimen, based on the results of the EMILIA trial [54]. In this study, women who had progressed on prior trastuzumab therapy were randomised to T-DM1 or lapatinib plus capecitabine. T-DM1 resulted in a longer median overall survival of 31 months compared to 25 months, and had lower rates of grade 3 or 4 toxicity, particularly diarrhoea and hand-foot syndrome, although thrombocytopenia and elevated liver enzymes were more common.

If not used in first line, T-DM1 should be considered standard second-line therapy [54]. Alternatively, it may also be employed in third or subsequent lines, as there is evidence of benefit in this setting from the TH3RESA trial. Compared to treatment of physicians' choice, T-DM1 led to a significant improvement in median progression-free and overall survival (6.2 versus 3.3 months and 22.7 versus 15.8 months, respectively) [55].

For subsequent lines of therapy, or as alternatives to the above regimens, a number of options exist. Trastuzumab is active in combination with a variety of single-agent chemotherapy, such as vinorelbine, taxane and capecitabine. Vinorelbine plus trastuzumab was compared to docetaxel plus trastuzumab in first line and showed equivalent progression-free and overall survival but significantly less treatment discontinuation due to toxicity [56]. Thus, its use in later lines of therapy should be similarly well tolerated. Furthermore, it represents a reasonable first-line therapy if pertuzumab is unavailable or taxanes are contraindicated. Given the lack of evidence supporting one combination over another, the choice should be made based on expected tolerability and patient preference.

Lapatinib plus capecitabine is another HER2-targeting combination, having been shown to be superior to capecitabine alone, modestly prolonging progression-free survival, with a trend to improving overall survival [57–58]. As a general alternative to trastuzumab, lapatinib is not preferred. When compared to trastuzumab plus paclitaxel in first line, lapatinib plus paclitaxel had a shorter time to progression and higher rates of toxicity [59]. Combining lapatinib and trastuzumab without chemotherapy improves overall survival compared to lapatinib alone in patients who have progressed on prior trastuzumab [60], and thus may be considered an alternative to trastuzumab plus chemotherapy, particularly in heavily pretreated patients with good performance status.

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Anti-HER2 Therapies in the Adjuvant and Advanced Disease Settings

47

Elisabetta Munzone

47.1 HER2-Positive Disease

Overexpression of human epidermal growth factor receptor type 2 (HER2, also referred to as HER2/neu or ErbB-2), a 185-kD receptor, was first described more than two decades ago [1]. Approximately 15–20% of breast cancers show amplification of the HER2 gene on chromosome 17 [2]. In the past, patients with HER2-positive breast cancer reported worse outcomes than did other patients with different subtypes of the disease [3]. Approval in 1998 of the first anti-HER2 agent (trastuzumab) led in a new era of molecularly targeted therapies for HER2-positive breast cancer and significantly improved outcomes in these patients [4].

Overexpression of HER2 in breast cancer is usually assessed by immunohistochemistry (IHC) or fluorescent in situ hybridization (FISH) on histopathological samples of primary cancer or metastatic tissue (Fig. 47.1). According to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP), HER2 positivity is defined by an IHC score of 3+, with strong staining of more than

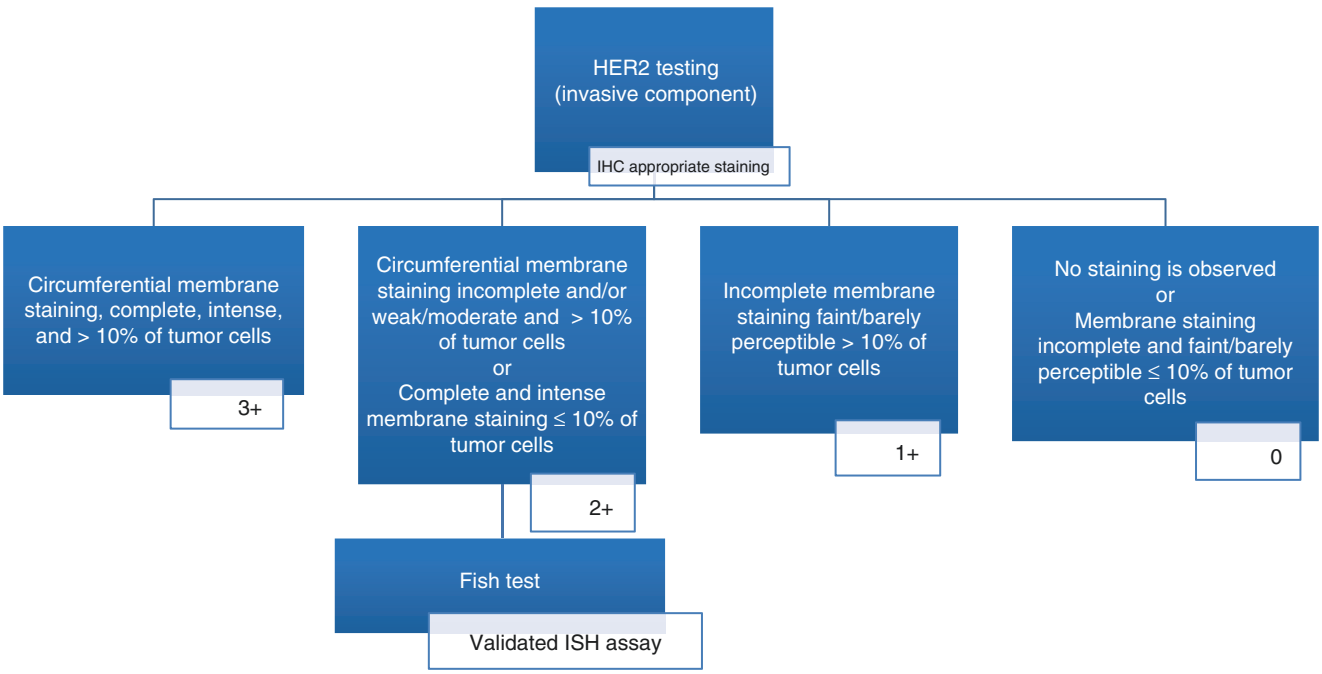

Fig. 47.1 Assessment of HER2 overexpression according to ASCO/CAP guidelines

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10% of all invasive tumor cells. An IHC score of 2+ needs further investigation, whereas IHC scores of 0 and 1+ are representative of HER2 negativity. FISH analysis can be performed as an additional determination after an IHC score of 2+ and also as a stand-alone test. FISH is based on the HER2 gene copy number, i.e., the ratio between the HER2 gene copy number and the number of chromosome 17 centromeres (CEP17). The ASCO/CAP define the presence of a HER2 amplification in FISH analysis as a dual-probe HER2/CEP17 ratio of ≥2.0 or a single-probe average HER2 copy number of ≥6.0 signals/cell [5].

47.2 HER2 Signaling and Overexpression

HER2 signaling promotes cell proliferation through the RAS–MAPK pathway and inhibits cell death through the phosphatidylinositol 3′-kinase (PI3K)–AKT–mTOR (mammalian target of rapamycin) pathway [6]. AKT includes three distinct enzymes, each of them is a member of the protein kinase family that is specific for serine–threonine and that inhibits apoptosis (programmed cell death); mTOR regulates the cellular functions that integrate upstream signaling inputs. Although HER2 overexpression has been described in a variety of human malignant conditions, gene amplification is usually rare except in breast cancer [7–12].

47.3 Currently Used HER2-Targeted Drugs

47.3.1 Trastuzumab

Trastuzumab is a humanized monoclonal antibody against the extracellular domain of the HER2 receptor [13].

The mechanism of action of trastuzumab may occur through both innate and adaptive immunities. Innate mechanisms lead to cell cycle arrest, with an increase in p27 levels, and decrease in cyclin D1 and cyclin-dependent kinase 2 activity [14]. Trastuzumab alone does not seem to promote a significant level of apoptosis, but it is synergistic with most chemotherapeutics in preclinical models. This synergism is felt in part to be explained by inhibition of the PI3K/AKT signaling pathway, which normally promotes cell survival [15]. Nevertheless, the innate mechanism alone does not fully explain the effect of trastuzumab on tumor regression. Adaptive mechanisms are also present, and preclinical models suggested that trastuzumab recruits immune effector cells that are responsible for antibody-dependent cell-mediated cytotoxicity (ADCC) [16]. This is likely to be T-cell mediated, through activation of the FC receptor, leading to increased cell death [17].

Several possible mechanisms by which trastuzumab might decrease signaling may include also prevention of HER2-receptor dimerization, increased endocytotic destruction of the receptor, inhibition of shedding of the extracellular domain, and immune activation [18].

Trastuzumab is approved for the treatment of early breast cancer overexpressing HER2 as part of a regimen consisting of anthracycline and sequential combination with either paclitaxel or docetaxel, or with carboplatin and docetaxel, or as a single agent following anthracycline-based therapy. Trastuzumab is approved for an overall duration of 12 months in the adjuvant setting (HERA trial). Trastuzumab is also approved for the treatment of metastatic breast cancer in combination with pertuzumab and taxanes for first-line treatment of HER2-overexpressing disease or in combination with other chemotherapeutic or endocrine agents for subsequent lines.

47.3.1.1 Subcutaneous Trastuzumab

The subcutaneous (SC) formulation of trastuzumab has been developed to provide an alternative to standard 3-weekly intravenous (IV) administration. The ready-to-use liquid SC formulation is injected as a fixed 600 mg dose in approximately 5 min [19].

The trastuzumab SC dose was selected based on nonclinical xenograft, pharmacology, and pharmacokinetics mouse and minipig studies. Initially a two-part phase I/Ib dose-finding/dose-confirmation study assessed trastuzumab SC dose according to body weight [20]; subsequently, pharmacokinetic modeling and simulation were used to determine a fixed (non-body-weight-adjusted) dose of trastuzumab SC that could be given without a loading dose. These analyses predicted that a fixed 600-mg dose of trastuzumab SC, administered every 3 weeks without a loading dose, would provide trastuzumab serum trough concentrations and exposure at least as high as those seen with the standard 3-weekly IV regimen. This fixed-dose trastuzumab SC regimen was then confirmed as appropriate in a phase III (neo)adjuvant randomized trial, the HannaH trial [21], which included 596 HER2-positive breast cancer patients. Trastuzumab SC was shown to be non-inferior to IV counterpart with regard to the co-primary end points. The SC and IV safety profiles were, in general, similar, with comparable distributions and types of adverse events (AEs), although a numerically higher proportion of serious AEs (SAEs) were reported with SC formulation. Updated safety data, along with efficacy data (EFS) from HannaH after a median follow-up of ~20 months, were subsequently reported and were consistent with the previously published data and the known safety profile of IV trastuzumab. EFS rates were comparable between the IV and SC groups [22].

SC and IV trastuzumab were also compared in the international, multicenter, open-label, randomized PrefHer study (n = 245 patients). PrefHer demonstrated that patients with HER2-positive early breast cancer (EBC) preferred SC over

IV administration, because it saved time and caused less pain, discomfort, and side effects [23]. PrefHer and HannaH confirmed SC trastuzumab as a validated and mostly preferred option for HER2-positive breast cancer patients.

47.3.2 T-DM1 (Ado-trastuzumab Emtansine)

Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate that is effective and generally well tolerated when administered as a single agent in advanced HER2-positive breast cancer patients. Efficacy has now been demonstrated in randomized trials as first-line, second-line, and later than the second-line treatment of advanced breast cancer.

T-DM1 is also an excellent example of a principle suggested almost 40 years ago to use antibodies as carriers of drugs to highly specific targets [24]. Antibody–drug conjugates (ADCs) are a means to deliver cytotoxic drugs specifically to cancer cells. The first ADC targeting the HER2 receptor is T-DM1 (ado-trastuzumab emtansine; T-MCC-DM1), which is a conjugate of trastuzumab and a cytotoxic moiety (DM1, derivative of maytansine).

T-DM1 has several mechanisms of action consisting of the antitumor effects of trastuzumab and those of DM1, a cytotoxic anti-microtubule agent released within the target cells upon degradation of the human epidermal growth factor receptor-2 (HER2)-T-DM1 complex in lysosomes [25, 26].

A key clinical trial to investigate the efficacy and safety of T-DM1 in the treatment of breast cancer was the EMILIA trial [27]. As patients assigned to T-DM1 lived longer (30.9 versus 25.1 months, respectively) and had fewer serious adverse events, T-DM1 was approved by the US Food and Drug Administration (FDA) in February 2013 for the treatment of patients with HER2-positive metastatic breast cancer who had previously received trastuzumab and a taxane. In the subsequent TH3RESA study, patients treated with T-DM1 achieved longer PFS (6.2 versus 3.3 months, respectively, hazard ratio 0.53, 95% CI 0.42–0.66) and longer survival (not reached versus 14.9 months) and had fewer severe adverse effects compared with a regimen chosen by the physician [28]. T-DM1 is currently being evaluated as adjuvant treatment for early breast cancer (NCT01853748, ATEMPT trial).

47.3.3 Pertuzumab

Pertuzumab is a recombinant humanized monoclonal antibody different from trastuzumab, because it is directed against the extracellular dimerization domain (subdomain II) of HER2, preventing dimerization of HER2 with other members of the HER family, such as HER3, HER1, and HER4 [29]. This results in inhibited downstream signaling of two key pathways that regulate cell survival and growth (the mitogen-activated protein kinase [MAPK] pathway and the phosphoinositide 3-kinase [PI3K] pathway), in addition to mediating antibody-dependent cell-mediated cytotoxicity. Pertuzumab is the first drug of a novel class of therapeutic antibodies known as HER dimerization inhibitors, which represent a complementary mechanism of action to trastuzumab. The addition of pertuzumab after progression to ongoing trastuzumab in xenografts was shown to synergistically increase tumor inhibition compared to trastuzumab treatment alone [30].

In combination with trastuzumab and docetaxel, pertuzumab was demonstrated to improve PFS and OS versus trastuzumab and docetaxel in the phase III CLEOPATRA trial in first-line HER2-positive metastatic breast cancer patients [31]. In a substudy of CLEOPATRA evaluating potential pharmacokinetic drug–drug interaction, pertuzumab showed no impact on the pharmacokinetics of trastuzumab or docetaxel [32].

Pertuzumab is the first drug to receive a fast-track approval from the US FDA, based on the pathologic complete response—as the primary end point—achieved in patients treated with neoadjuvant chemotherapy for breast cancer. Pertuzumab is also approved as first-line treatment in metastatic setting both by FDA and EMA in combination with trastuzumab and docetaxel.

47.3.4 Lapatinib

Lapatinib is a dual EGFR/HER2 tyrosine kinase inhibitor that binds to the intracellular ATP-binding pocket of the protein kinase domain of HER2. By binding, lapatinib prevents autophosphorylation of the cytoplasmic domain and thereby downstream signaling and tumor cell growth [33]. Lapatinib reduces EGFR and HER2 signaling and induces apoptosis in multiple models of HER2-overexpressing breast cancer [34].

In 2007, the US Food and Drug Administration approved lapatinib for use in combination with capecitabine for the treatment of women with HER2-overexpressing, advanced, or metastatic breast cancer after cytotoxic drugs or trastuzumab. In 2010, the EMEA approved lapatinib with letrozole in postmenopausal HER2-positive, hormone receptor-positive breast cancer for the first-line treatment of postmenopausal women [35].

The registration of lapatinib was approved primarily based on a phase III, randomized, open-label study comparing lapatinib plus capecitabine with capecitabine alone in 324 patients with HER2-positive advanced breast cancer or metastatic breast cancer that had progressed during prior treatment with anthracyclines, taxanes, and trastuzumab [36].

47.4 Adjuvant Anti-HER2 Treatments

Current clinical guidelines clearly state that standard of care in 2015 recommends the use of the monoclonal anti-HER2 antibody trastuzumab in combination with or after adjuvant chemotherapy in medically fit patients diagnosed with stage I–III HER2-positive breast cancer [37, 38].

Guidelines are based on the results of six phase III randomized trials published or reported so far, exploring the benefit of adding trastuzumab to adjuvant chemotherapy for early HER2-positive breast cancer patients (Table 47.1).

The herceptin adjuvant trial (HERA trial) enrolled 5090 women with HER2-positive early breast cancer after completion of locoregional therapy (surgery ± radiotherapy) and at least four courses of chemotherapy. Patients were randomized to either a control group or 1 or 2 years of treatment with trastuzumab. A first analysis—after 1 year of median follow-up—indicated the benefit of trastuzumab in adjuvant systemic therapy with an unadjusted hazard ratio (HR) of 0.54 for an event in the trastuzumab group (1 year) compared to the observation group [39]. A final comparison of the interventional arms showed no evidence of better outcome with 2 years versus 1 year of trastuzumab. Nevertheless, the number of adverse cardiac events slightly rose through 2 years of drug administration compared to 1 year. The HR for disease-free survival (DFS) and also for overall survival (OS) was 0.76 each, for 1 year of trastuzumab treatment versus observation, which confirms the efficacy of trastuzumab therapy [40].

The results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-31 trial and the North Central Cancer Treatment Group (NCCTG) N9831 trial were presented together in one analysis. In both trials, 4046 women with HER2-positive early-stage breast cancer were treated with chemotherapy consisting of four cycles of doxorubicin and cyclophosphamide (AC) followed by paclitaxel for 3 months (weekly or q21d). The NSABP B-31 study randomized patients to chemotherapy with or without 52 weeks of trastuzumab administration afterward. The NCCTG N9831 study had three arms: chemotherapy only, chemotherapy plus concurrent trastuzumab, and paclitaxel or plus sequential trastuzumab after paclitaxel. The interim and final analyses of the trials demonstrated the benefit of adding trastuzumab for DFS and OS, by increasing the 10-year OS rate from 75.2% to 84%, thus leading to a 37% relative improvement in OS (HR 0.63, $P < 0.001$). The DFS benefits from trastuzumab showed an increase of the 10-year DFS rate from 62.2% to 73.7%. Thus, HER2 blockade resulted in a 40% (HR 0.60, $P < 0.001$) improvement of the DFS [41, 42]. Further analysis in the NCCTG N9831 study was done regarding the efficacy of concurrent versus sequential administration of trastuzumab. Although the analysis results were not statistically significant, they showed a trend of a prolonged DFS in the concurrent treatment arm (concurrent/sequential HR 0.77; 99.9% confidence interval (CI), 0.53–1.11, $P = 0.02$) and supported the recommendation of a concurrent regimen of trastuzumab with taxane [43].

Another adjuvant trial was the Breast Cancer International Research Group (BCIRG)-006 study (NCT00021255), which investigated the efficacy of trastuzumab as well as the effectiveness and safety of an anthracycline-free chemotherapy regimen in order to reduce cardiac toxicity. After randomization, 3222 women with HER2-positive early-stage high-risk breast cancer were treated with doxorubicin, cyclophosphamide, docetaxel (ACT), ACT plus trastuzumab for 1 year (AC-TH), or with the non-anthracycline regimen consisting of docetaxel, carboplatin, and 52 weeks of trastuzumab (TCH). The final analysis confirmed the importance of trastuzumab treatment, with superiority of both the trastuzumab-containing regimes compared to ACT (OS 87%, DFS 75%). No significant difference was observed in

Table 47.1 Adjuvant trastuzumab trials

Trial	Median FU (years)	*N* Patients	Treatament	DFS: HR (95% CI)	DFS	OS:HR (95% CI)	OS
HERA	8	5099	CT vs. CT ➔ T	0.76 (0.67–0.86)	70% vs. 76%	0.76 (0.65–0.88)	84.5% vs. 87%
NSABP B-31	8.4[a]	4046[a]	AC ➔ P vs. AC ➔ PT ➔ T	0.60 (0.53–0.68)	62.2% vs. 73.7%	0.63 (0.54–0.73)	75.2% vs. 84%
NCCTG N9831	8.4[a]		AC ➔ P vs. AC ➔ P ➔ T vs. AC ➔ PT ➔ T				
BCIRG 006	5.5	3222	AC ➔ D vs. AC ➔ DT vs. DCarboT	0.64 (0.53–0.78)	75% vs. 84%	0.63 (0.48–0.81)	87% vs. 92%
				0.75 (0.63–0.90)	81%		91%
PACS-04	3.9	528	FEC/ED vs. FEC/ED ➔ T	0.86 (0.61–1.22)	78% vs. 81%	1.27 (0.68–2.38)	96% vs. 95%
FinHER	5.2	112	D ➔ FEC vs. DT ➔ FEC	0.32 (0.12–0.89)	74.1% vs. 92.5%	0.42 (0.13–1.33)	82% vs. 94.4%

[a]Joint analysis

CT chemotherapy, *T* trastuzumab, *AC* doxorubicin Cyclophosphamide, *P* paclitaxel, *D* docetaxel, *Carbo* carboplatin, *FEC* fluorouracil epirubicin cyclophosphamide, *ED* epirubicin docetaxel

OS and DFS for AC-TH (OS 92%, DFS 84%) and TCH (OS 91%, DFS 81%). A trend for better outcome occurred in the AC-TH arm compared to TCH (124 vs. 144 distant relapse events). Cardiotoxicity was lower in the TCH arm. Especially for patients with high risk of cardiac toxicity, the TCH regimen may be considered as an appropriate alternative [44].

The FinHER study analyzed 1010 patients randomized to receive adjuvant docetaxel or vinorelbine followed by FEC regimen. Patients with an amplified HER2/neu gene ($n = 232$) were further randomized to receive weekly trastuzumab for 9 weeks, concurrently to docetaxel/vinorelbine or not. At a median follow-up of 36 months, the inclusion of trastuzumab in the adjuvant chemotherapeutic regimen resulted in a significantly better DFS (HR 0.42 95% CI, 0.21–0.83; $p = 0.01$); also, OS tended to be better, although not significant ($p = 0.07$) [45]. The last update of the study, at a median follow-up of 5 years, confirmed the benefits of adding a short course of concurrent trastuzumab to docetaxel followed by FEC versus chemotherapy alone (HR for distant disease recurrence 0.32, $p = 0.029$) [46]. However, due to the limited sample size, these results need to be confirmed in larger series.

Recently, the PHARE trial further explored the question of the optimum duration of adjuvant trastuzumab treatment. In total, 3381 patients with HER2-positive early breast cancer who had received at least four cycles of chemotherapy and up to 6 months of trastuzumab prior to randomization were randomized to continue trastuzumab for a total of 12 or 6 months [47]. This was a non-inferiority study, aiming to prove that the shorter course of trastuzumab is not inferior to standard 1 year of treatment. The study did not meet its primary end point; after a median follow-up of 42.5 months, 175 and 219 DFS events were observed in the 12-month and in the 6-month group, respectively, with the 2-year DFS rates being 93.8% (95% CI 92.6–94.9) and 91.1% (89.7–92.4), respectively (HR 1.28, 95% CI 1.05–1.56; $p = 0.29$). The 6-month trastuzumab duration was associated with fewer cardiac events as compared to the 12-month group (32 of 1690 patients vs. 96 of 1690 patients, respectively, $p < 0.0001$).

In the PACS-04 study, after a first randomization between epirubicin docetaxel and FEC, HER2-positive patients ($n = 528$) were randomly assigned to receive sequential trastuzumab or to observation. At a median follow-up of 4 years, the addition of sequential trastuzumab failed to detect a significant reduction in the risk of recurrence or death [48].

The Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO) trial started with the aim to analyze the efficacy of the combination therapy in adjuvant treatment. The trial was the largest study on adjuvant therapy in HER2-positive breast cancer enrolling more than 8000 participants. Women were treated with surgery and chemotherapy (anthracycline based vs. anthracycline-free), followed by randomization to trastuzumab alone, lapatinib alone, trastuzumab followed by lapatinib, or concurrent therapy with lapatinib and trastuzumab. Anti-HER2 therapy was given for 52 weeks and was combined with paclitaxel/docetaxel in women with prior anthracycline-based chemotherapy for the first 12 weeks. The lapatinib arm was prematurely closed since an interim analysis in 2011 suggested inferiority of this arm. First results were presented at the ASCO Annual Meeting in 2014 [49]. After 4 years of follow-up, the DFS rate for trastuzumab plus lapatinib was 88% compared to 86% for trastuzumab monotherapy (HR 0.84, 97.5% CI 0.7–1.02, $p = 0.048$) and 87% for trastuzumab followed by lapatinib versus 86% for trastuzumab monotherapy (HR 0.96, 97.5% CI 0.8–1.25, $p = 0.610$). In addition, the combination of trastuzumab and lapatinib showed no benefit regarding OS compared to trastuzumab. Adverse events were more likely in patients within the lapatinib arm; the overall cardiac toxicity was tolerable.

47.4.1 Small HER2-Positive Tumors

Retrospective evidence from institutional series of reference cancer centers or country databases [50, 51] and also subgroup analysis of one of the major pivotal trials [44] (e.g., BCIRG 006) suggest a benefit for the administration of trastuzumab compared to the nonuse of trastuzumab that may still warrant the risks for "small" T1a,bN0M0 tumors.

In a retrospective series on 16,975 consecutive patients with T1a/b HER2-positive disease, 5-year invasive distant recurrence-free interval was 99.0% for T1a patients and 97.0% for T1b patients [52].

These small tumors indeed have an adverse prognosis when untreated and remain at significant risk of relapse when treated with chemotherapy only. The NCCN Guidelines Version 1.2016 Breast Cancer available at http://www.nccn.org/professionals/ physician_gls/pdf/breast.pdf and the St. Gallen Guidelines [37] do recommend administering adjuvant trastuzumab for early breast cancer patients with T1b tumors (>0.5 and 1.0 cm) and lymph node negative.

For an evaluation of single standard treatment in patients with small ($T < 3$ cm), node-negative HER2-positive breast cancer, Tolaney et al. [53] recruited 406 patients in a single-group study. Women were treated with paclitaxel and trastuzumab for 12 weeks, followed by trastuzumab monotherapy for 40 weeks. The 3-year rate of survival free from invasive disease was 98.7% (95% CI 97.6–99.8), and the 3-year rate of recurrence-free survival was 99.2% (95% CI 98.4–100). With a risk of early recurrence of about 2% and a low rate of serious toxic effects (symptomatic congestive heart failure 0.5%), treatment with adjuvant paclitaxel and trastuzumab could be considered as an option for node-negative, small HER2-postive breast cancer.

The excellent disease-free survival observed with adjuvant paclitaxel and trastuzumab without anthracyclines highlighted the possibility to de-escalate treatment for low-risk, early-stage, HER2-positive disease.

47.5 Neoadjuvant Anti-HER2 Trials

The main candidates for neoadjuvant treatment in the HER2-positive setting are patients with a high likelihood of achieving a pCR. A recent meta-analysis, from the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC), included 11,955 patients treated in 12 randomized neoadjuvant trials [54]. Results indicated that patients who obtain a pCR, defined as either the absence of invasive and in situ carcinoma in breast and axilla or only invasive carcinoma in both breast and axilla, have a more favorable long-term outcome than those without a pCR (HR for OS = 0.36 with both definitions, HR for event-free survival [EFS] = 0.44 with ypT0 ypN0, and HR = 0.48 with ypT0/Tis ypN0) [54].

The Early Breast Cancer Trialists' Collaborative Group overview of neoadjuvant trials demonstrated that standard chemotherapy regimens (anthracyclines/taxanes) whether given preoperatively or postoperatively provided the same long-term clinical outcomes. Therefore, one can now potentially deliver standard systemic treatment in either the neoadjuvant or adjuvant setting with similar confidence [55]. The same is true, if not even more so, for HER2-positive breast cancers. The additional advantages of delivery in the neoadjuvant setting include the ability to study new agents with the utility of a surrogate end point for outcome; the ability to obtain tumor tissue for pharmacodynamic assessment, understanding of biology, and discovery of predictive biomarkers; earlier initiation of systemic therapies; and the ability to monitor response (which is clearly not possible in the adjuvant setting).

In an early MD Anderson trial comparing trastuzumab-containing therapy versus chemotherapy alone, the pCR rate was doubled (67% versus 25%) with trastuzumab, leading to early termination of the trial (Table 47.2) [56].

One of the first trials underlining the concept of dual blockade was the phase III GeparQuinto trial (NCT00567554), in which the addition of trastuzumab versus lapatinib to anthracycline–taxane-based neoadjuvant chemotherapy was tested in 620 women with operable or locally advanced HER2-positive breast cancer. Patients were treated either with epirubicin, cyclophosphamide and docetaxel plus trastuzumab, or the same chemotherapy regimen plus lapatinib for a total of 6 months and underwent surgery afterward. The trastuzumab arm showed a significantly better pathologic complete response (pCR) rate, with 30.3% versus 22.7% in the lapatinib group [57]. Consequently, the authors

Table 47.2 Efficacy data from main anti-HER2 neaoadjuvant trials

Trial	*N* Patients	Treatment	pCR (Breast and nodes)	*P*	3-Year EFS
NOAH	235	CT alone vs. CT + H	19.5% vs. 38.5%	0.001	43% vs. 58%[a]
GeparQuinto	620	ECH ➔ TH vs. ECL ➔ TL	31.3% vs. 21.7%	0.05	NA
NEOALTTO	455	H ➔ HP vs.	27.6% vs.		76%
		L ➔ LP vs.	20% vs.	0.13	78%
		HL ➔ HLP	46.9%	0.001	84%
Cher-LOB	121	HP ➔ FECH	25%		
		LP ➔ FECL	26.3%	0.19	NA
		HLP ➔ FECHL	46.7%		
NSABP B41	519	AC ➔ HP	52.5% (b)		
		AC ➔ LP	53.2% (b)	0.9852	NA
		AC ➔ HLP	62% (b)	0.095	
CALGB 40601	305	HP	40% (b)		
		LP	32% (b)		NA
		HLP	51% (b)	0.11	
NeoSphere	417	TH	29% (b)		81%[a]
		PerHT	45.8% (b)	0.0141	86%
		PerH	24% (b)		73%
		PerT	16.8% (b)		73%
TRYPHAENA	225	PerHFEC ➔ PerTH	61.6% (b)		
		FEC ➔ PerTH	57.3% (b)	NA	NA
		TcarboHPer	66.2% (b)		

b breast, *CT* chemotherapy, *H* trastuzumab, *AC* doxorubicin Cyclophosphamide, *P* paclitaxel, *D* docetaxel, *Carbo* carboplatin, *FEC* fluorouracil epirubicin cyclophosphamide, *L* lapatinib, *Per* pertuzumab

[a]5-years EFS

concluded that outside clinical trials, lapatinib should not be used as single anti-HER2 treatment in combination with neoadjuvant chemotherapy.

The randomized phase III NOAH trial conducted by Gianni and colleagues confirmed the significant pCR and EFS benefit of combining trastuzumab with neoadjuvant chemotherapy and continuing adjuvant trastuzumab after surgery in HER2-positive disease [58]. Furthermore, it showed that combining trastuzumab with anthracycline-based chemotherapy is tolerable and is not associated with an increase in cardiac toxicity. Updated NOAH results were presented at the 2013 American Society of Clinical Oncology (ASCO) Annual Meeting. After a median follow-up of 5.4 years, the EFS benefit with trastuzumab was confirmed, and a strong trend toward improved OS was observed. Cardiac tolerability was good despite concurrent administration of trastuzumab with doxorubicin [59]. Accordingly, a more recent small study including only 80 patients showed a 92.9% 4-year RFS in patients who achieved pCR after trastuzumab-based neoadjuvant treatment versus 72.4% without pCR. All cases of symptomatic cardiotoxicity were resolved during follow-up [60]. On the basis of the NOAH results, the European Medicines Agency (EMA) extended the approved indication for trastuzumab to include its use in combination with neoadjuvant chemotherapy followed by adjuvant trastuzumab monotherapy for locally advanced and inflammatory HER2-positive disease or for tumors >2 cm in diameter. The impressive results observed with trastuzumab in the neoadjuvant setting led to the initiation of several trials evaluating new HER2-targeted agents with or without trastuzumab.

Subsequently, the NSABP B-41 and the NeoALTTO trials compared trastuzumab single agent, lapatinib single agent, and the dual HER2 blockade by combination of trastuzumab plus lapatinib. In these studies, the substitution of lapatinib for trastuzumab in combination with chemotherapy resulted in similarly high pCR rates (52.5% with trastuzumab versus 53.2% with lapatinib, $p = 0.985$) [61, 62]. Furthermore, in the long follow-up of the NeoALTTO trial, neither EFS nor OS did differ between the lapatinib and trastuzumab groups (EFS, HR 1.06, $p = 0.81$; OS, HR 0.86, $p = 0.65$) [63].

However, although the combination of trastuzumab and lapatinib plus chemotherapy led to a higher pCR rate compared to anti-HER2 single-agent treatment in the NeoALTTO trial (51.3% with combination versus 29.5% with single agent; $p = 0.0001$), it did not reach statistical significance in the NSABP B-41 (pCR 52.5% with trastuzumab versus 62.0% with combination, $p = 0.095$). The same approach with double HER2 inhibition has been explored in the randomized phase II CHER-LOB trial [64], confirming the potential role of lapatinib associated with trastuzumab.

The Cancer and Leukemia Group B 40601, a neoadjuvant phase III trial of weekly paclitaxel (T) and trastuzumab (H) with and without lapatinib (L) in HER2-positive breast cancer, was presented at the 2013 ASCO Annual Meeting [65]. This trial randomly selected 305 patients, of which two thirds had clinical stage II disease. The pCR rates in the breast alone were 51% (42–60%) in the THL arm, 40% (32–49%) in the TH arm, and 32% (22–44%) in the TL arm. The combination arm of THL was not significantly different from the standard arm of trastuzumab and paclitaxel ($p = 0.11$).

In the NeoSphere trial [66], the combination of trastuzumab, pertuzumab, and docetaxel demonstrated a significantly higher pCR rate than with trastuzumab and docetaxel alone (pCR 29.0% with docetaxel plus trastuzumab, 45.8% with docetaxel plus trastuzumab and pertuzumab, 16.8% with trastuzumab and pertuzumab alone, and 24.0% with docetaxel and pertuzumab), without substantial differences in tolerability. These findings justified the EMA approval of pertuzumab as primary systemic therapy for HER2-positive early breast cancer.

Moreover, the TRYPHAENA phase II randomized trial suggested that combining pertuzumab and trastuzumab with standard neoadjuvant chemotherapy posed no cardiac safety concerns, and it demonstrated a pCR rate higher than 66% with an anthracycline-free regimen [67].

Taking into account the results of all these clinical trials, neoadjuvant treatment with anti-HER2 targeted agents should be offered to patients with HER2-positive locally advanced or inflammatory breast cancer.

47.6 Treatment of HER2-Positive Metastatic Breast Cancer

The landscape of HER2-positive metastatic BC treatment is evolving rapidly. In the past decade, first-line trastuzumab combined with a taxane (paclitaxel or docetaxel) or vinorelbine has been considered the most active and effective treatment choice for HER2-positive metastatic breast cancer, with response rates of 50–60% and median time to progression (TTP) ranging from 7 to 15 months [68, 69]. After disease progression on first-line trastuzumab-based therapy, continued HER2 blockade with lapatinib plus capecitabine [70, 71] or trastuzumab plus capecitabine [72] was considered a standard in clinical practice.

In postmenopausal patients, chemotherapy-free therapeutic options were successfully explored in patients with highly endocrine-sensitive non-life-threatening and/or slowly progressive disease. A chemotherapy-free first-line regimen of trastuzumab plus anastrozole demonstrated a significant improvement in PFS (median 5 versus 2 months, respectively) and response rate (20% versus 7%, respectively) com-

pared with anastrozole alone [73]. Similar results were obtained with lapatinib plus letrozole (median PFS 8 versus 3 months, respectively, $p = 0.019$, clinical benefit rate 48% versus 29%, respectively, $p = 0.003$) [74].

Starting from 2012, a paradigm shift was observed in the management of HER2-positive metastatic breast cancer following the results from the CLEOPATRA [31], the EMILIA [27], and the TH3RESA trials [28]. CLEOPATRA was a double-blind placebo-controlled phase III trial comparing the combination of trastuzumab and docetaxel plus pertuzumab or placebo as first-line treatment in 808 patients with HER2-positive metastatic breast cancer [31]. At the first analysis, the addition of pertuzumab significantly improved progression-free survival (PFS 18.5 months with pertuzumab-containing therapy versus 12.4 months in the control arm, HR 0.62, 95% CI 0.51, 0.75; $p < 0.001$). The response rate was 80% versus 69%, respectively ($p = 0.001$), and a strong trend toward an OS improvement was shown with the combination of pertuzumab, trastuzumab, and docetaxel. Lately, after a median follow-up of 50 months, the final analysis showed 16 months improvement in OS (56.5 months in the pertuzumab arm versus 40.8 months in the placebo arm, HR = 0.68; $p = 0.0002$) [75]. The main criticism of the trial was that only a minority of patients had previously received trastuzumab (11% of patients) or a taxane (23% of patients) in the neo/adjuvant setting. This characteristic of the patients' population was noticed as possibly underrepresentative of everyday clinical practice. However, subgroup analyses suggested a similar benefit from pertuzumab-containing therapy irrespective of prior neo/adjuvant chemotherapy (HR 0.61 in patients who had received neo/adjuvant chemotherapy versus 0.63 in those who had not). Pertuzumab did not add to trastuzumab cardiotoxicity. The phase III EMILIA trial compared trastuzumab-emtansine (T-DM1) with lapatinib plus capecitabine in 991 patients with HER2-positive metastatic BC who were previously treated with a taxane and trastuzumab [27]. T-DM1 was associated with significantly improved PFS (9.6 months with T-DM1 versus 6.4 months with lapatinib plus capecitabine; HR 0.65, 95% CI 0.55–0.77, $p < 0.001$), OS (30.9 versus 25.1 months, respectively; HR 0.68, 95% CI 0.55–0.85, $p < 0.001$), and response rate (44% versus 31%, respectively; $p < 0.001$). Nevertheless, the benefit of T-DM1 was observed even in patients whose disease progressed less than 6 months after completing neo/adjuvant trastuzumab. Furthermore, T-DM1 was better tolerated than lapatinib and capecitabine. The phase III TH3RESA trial randomized patients with progressive disease after two or more HER2-targeted regimens for metastatic HER2-positive breast cancer. Patients received T-DM1 or physician's choice therapy [28]. Median PFS was significantly improved with T-DM1 compared with physician's choice (6.2 months versus 3.3 months, HR 0.528, $p < 0.0001$). Interim OS analysis showed a trend favoring T-DM1 (HR 0.552, 95% CI 0.369–0.826, $p = 0.0034$), but the stopping boundary was not crossed. Moreover, a lower incidence of grade 3–4 adverse events was reported with T-DM1 than with physician's choice. Following these trials, in 2013, T-DM1 was approved by EMA as a single agent for the treatment of HER2-positive, unresectable locally advanced, or metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination. As the efficacy of these first- and second-line therapies in HER2-positive metastatic breast cancer is substantial, oncologists have now an improved and challenging armamentarium for managing patients whose disease progresses after two lines of HER2-targeted therapy.

The combination of lapatinib plus trastuzumab in the EGF104900 trial [76] demonstrated improved OS compared with lapatinib alone in patients with heavily pretreated disease (median three prior lines of trastuzumab). The HR for OS was 0.74 (95% CI 0.57–0.97; $p = 0.026$), and the 1-year OS rate was 56% with the combination versus 41% with lapatinib alone. An exploratory analysis showed that combining the two anti-HER2 agents was particularly effective in subgroups of patients with ER/PgR negative tumors or treated with less than four prior lines of trastuzumab. However, in Europe, the combination of lapatinib plus trastuzumab is not approved, and the current practice in patients whose disease progresses on trastuzumab plus a taxane or on lapatinib plus capecitabine is to rechallenge with trastuzumab combined with an alternative chemotherapy, even though the evidence supporting this approach is derived only from retrospective studies [77].

Finally, the phase III study MARIANNE recently evaluated the efficacy of pertuzumab and T-DM1 compared to T-DM1 alone or trastuzumab with taxane in the frontline HER2-positive, metastatic, or locally advanced setting [78]. During the 2015 ASCO meeting, first results in 1095 patients were presented. T-DM1 treatment resulted in non-inferior, but not superior, progression-free survival (PFS) compared with trastuzumab plus a taxane (HT). Therefore, in this setting, the addition of pertuzumab to T-DM1 provided no efficacy benefit.

To summarize, following the remarkable results of recent first- and second-line trials in HER2-positive metastatic breast cancer, the optimal treatment algorithm has been revisited (Fig. 47.2). At the third disease progression in a patient still fit enough for active treatment, enrollment in a clinical trial is warranted; alternatively, rechallenge with trastuzumab combined with a non-cross-resistant chemotherapy or with single-agent anthracycline therapy may be considered.

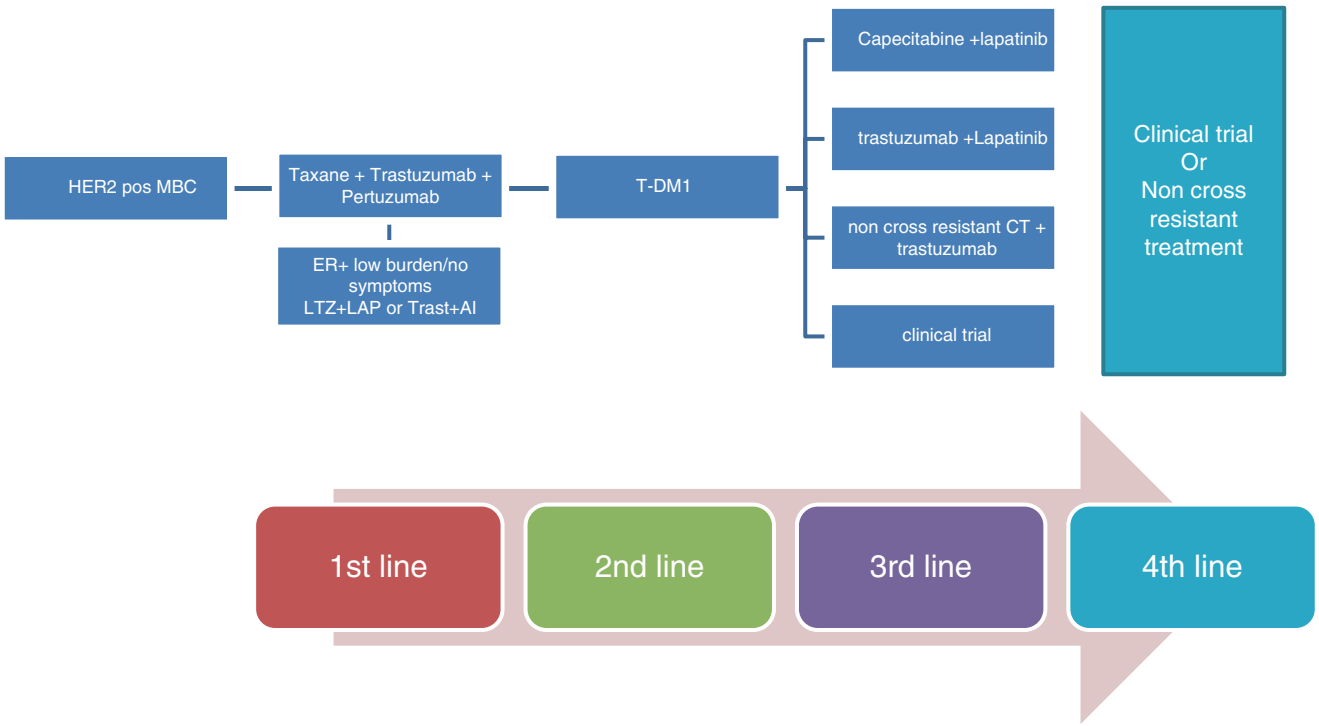

Fig. 47.2 Proposed treatment algorithm for HER2-positive MBC

47.6.1 Continuing Trastuzumab Beyond Progression

The current practice of continuing trastuzumab until progression in responding patients is based on two phase III trials, and long-term trastuzumab treatment does not appear to be associated with an excess in cardiac toxicity [79].

The German Breast Group 26 study enrolled 156 pretreated patients, the majority of whom had already received trastuzumab for metastatic disease [72]. Patients were randomized to capecitabine (1250 mg twice daily for 14 days every 3 weeks) plus trastuzumab (6 mg/kg every 3 weeks) or to capecitabine alone. With a median follow-up of 15.6 months, the addition of trastuzumab to capecitabine was associated with improvements in TTP (8.2 months vs. 5.6 months; HR, 0.69) and response rate (48% vs. 27%). Although OS was better with the combination, the effect was not statistically significant (25.5 months vs. 20.4 months, $p = 0.257$). The combination arm was not associated with increased toxicity, except for anemia (64% vs. 42%).

Several retrospective analyses of trastuzumab continuation beyond progression were published over the years [80–82]. These studies share the common limitations of a retrospective approach. However, it seems highly unlikely that there will be further prospective, randomized studies examining the specific role of trastuzumab combined with further-line chemotherapy for women who have progressed on trastuzumab. The international oncological community has already adopted the practice of continuing trastuzumab beyond progression. Nevertheless, until novel anti-HER2 therapies establish their role, continuation of trastuzumab may remain an option in the management of patients with HER2-positive advanced breast cancer.

47.7 New Anti-HER2 Drugs

47.7.1 Afatinib

Afatinib is an oral small molecule that irreversibly inhibits HER1, HER2, and HER4 [83]. A phase II study in trastuzumab-resistant metastatic patients showed initial responses [84]. Adverse events included diarrhea and rash. LUX-breast 1 is a phase III study of vinorelbine plus trastuzumab or afatinib for metastatic patients who progressed to one chemotherapy regimen containing trastuzumab (NCT01125566) [85]. As of April 2013, 508 patients were randomized. Both arms demonstrated similar PFS and ORR, but OS diverged and was shorter for the afatinib-containing arm compared to the trastuzumab arm. The safety profile of afatinib and vinorelbine was consistent with the individual monotherapies, but its tolerability compared unfavorably to trastuzumab and vinorelbine.

LUX-breast 2 is an ongoing phase II trial (NCT01271725) investigating efficacy and safety of afatinib alone (40 mg/day) followed by afatinib "beyond progression" plus chemo-

therapy. LUX-breast 3 is a randomized phase II study of afatinib alone or with vinorelbine versus investigator's choice of treatment in patients with HER2-positive breast cancer and with progressive brain metastases after trastuzumab or lapatinib-based therapy.

47.7.2 Neratinib

Neratinib is another oral, irreversible inhibitor of HER1, HER2, and HER4 [86]. A phase II trial evaluated neratinib in 136 HER2-positive patients [87] in either pretreated or trastuzumab-naïve patients. Median PFS were 22.3 and 39.6 weeks and ORR were 24% and 56%, respectively. Diarrhea was the most common grade 3/4 adverse effect. Another phase I–II trial combined neratinib plus trastuzumab in 45 metastatic and trastuzumab-resistant patients showed an encouraging 27% ORR [88]. Finally, a phase I–II trial evaluated neratinib plus vinorelbine in trastuzumab- or lapatinib-pretreated patients (n = 77) [89]. ORR was 42% in lapatinib-treated and 51% in lapatinib-naïve patients. Open-label phase II trials are currently testing neratinib monotherapy in patients with HER2-positive metastatic brain tumors (NCT01494662). Also a phase III trial (ExteNET) in the adjuvant setting is ongoing (NCT00878709). First results in 2840 women with HER2-positive breast cancer and prior adjuvant trastuzumab and chemotherapy were recently presented [90]. Patients were randomly assigned to receive either 240 mg/day of neratinib for 1 year or placebo (1420 patients in each arm). At 24 months, patients who received neratinib had an iDFS rate of 93.9% compared to 91.6% in the placebo group (hazard ratio [HR] 0.67, 95% CI [0.50, 0.91]; p = 0.009). As previously reported, diarrhea was the most common adverse event with neratinib; grade 3/4 diarrhea occurred in 39.9% of patients compared with 1.6% of patients who received placebo.

47.7.3 MM-111

MM-111 is a bi-specific monoclonal antibody that reversibly targets the HER2 and HER3 heterodimer [91]. A phase I–II study is currently evaluating its efficacy as single agent in HER2-positive advanced breast cancer patients who have received prior trastuzumab or lapatinib therapy (NCT00911898) [92]. Another phase I trial is studying MM-111 plus trastuzumab in HER2-positive, heregulin-positive, advanced, and refractory breast cancer (NCT01097460).

47.7.4 HER2-Targeted Vaccines

Cancer vaccines designed to induce specific anti-HER2 immunity are under investigation. Different strategies include protein-based vaccines, plasmid DNA-based vaccines, and vaccines that deliver HER2 in a viral vector. HER2 peptide-based vaccines were tested in patients with metastatic HER2-positive breast cancer [93, 94]. Patients immunized developed delayed-type hypersensitivity reactions and strong CD8+ cell responses specific for HER2 [95]. Among dendritic cell-based vaccine, a small group of patients with stage IV breast cancer [96] showed initial promising responses. More recently, cell-based GM-CSF-secreting vaccines are being tested in combination with trastuzumab [97, 98], and several clinical trials are underway.

The induction of a stable and strong immunity by cancer vaccines is expected to lead to establishment of immune memory, thereby preventing tumor recurrence. However, an immunological tolerance against HER2 antigen exists representing a barrier to effective vaccination against this oncoprotein. As a consequence, the current challenge for vaccines is to find the best conditions to break this immunological tolerance [99].

Several approaches have been proposed, including vaccines coupled to inhibitory molecules, monoclonal antibodies, bacteria or bacterial compounds, radiotherapy, and chemotherapy. However, the best strategy still represents a challenge.

47.7.5 PI3K/AKT/mTOR Blocking Drugs

PI3K/AKT/mTOR is an intracellular signal pathway frequently deregulated in breast cancer and involved in primary or secondary resistance to anti-HER2 agents [100]. A phase I study tested the combination of everolimus plus weekly paclitaxel and trastuzumab in 33 patients with heavily pretreated metastatic disease [101]. Encouraging activity was reported, with an overall disease control rate at 6 months of 74%. BOLERO-3 trial explored the addition of vinorelbine to everolimus plus trastuzumab in previously treated patients. With 569 patients completing the BOLERO-3 study, median PFS was 7.0 vs. 5.78 months in the placebo arm (p = 0.0067) [102].

The addition of everolimus to weekly trastuzumab plus paclitaxel in the first-line metastatic breast cancer setting did not improve outcomes in the phase III BOLERO-1 trial, but provided a hint of efficacy in the hormone receptor-negative subset [103]. The study enrolled 719 patients with locally advanced or metastatic HER2-positive breast cancer and no prior treatment in this setting. They were randomly assigned 2:1 to everolimus (10 mg/day) plus paclitaxel and trastuzumab or paclitaxel/trastuzumab alone, until disease progression or intolerable toxicity.

In the full study population, progression-free survival was comparable between the arms: 14.95 months with the addition of everolimus and 14.49 months with placebo (hazard ratio [HR] = 0.89, p = .1166). In the hormone receptor-negative subpopulation, however, everolimus-treated patients achieved a median progression-free survival of 20.27 months vs. 13.08 months with placebo (HR = 0.66, p = .0049).

47.7.6 Heat Shock Protein 90 Pathway

Heat shock protein 90 (Hsp-90) is a molecular chaperone that provides stability and supports the functionality of several proteins. Many of these proteins (i.e., Bcr-Abl, c-Kit, and PDGF-α) are pro-oncogenic. Inhibition of Hsp-90 degrades HER2 and Hsp-90 inhibitors have shown activity in HER2-driven xenograft models.

Concerning HER2-positive breast cancer, it is known that Hsp-90 is required for the stabilization of essential components of EGFR and HER2 signaling (HER2, AKT, c-SRC, RAF, and HIF-1α) [104]. Clinical data from a phase I trial with the Hsp-90 inhibitor tanespimycin used in combination with trastuzumab as second-line therapy showed evidence of antitumor activity in 63% of patients [105]. A phase II trial in 31 patients with HER2-positive metastatic breast cancer whose disease has progressed on trastuzumab further confirmed that tanespimycin plus trastuzumab has significant anticancer activity in this category of patients. The ORR was 22% and the clinical benefit reached 59% [106].

47.7.7 Other Exploratory Anti-HER2-Blocking Strategies

Trials combining anti-HER2 agents with drugs blocking different signaling pathways are ongoing trying to address further improvement. A promising approach seems to be the combination of anti-HER2 therapy with insulin growth factor receptor (IGFR-1) blocking agents. IGFR-1 inhibition has been shown to restore sensitivity to trastuzumab in animal models [107]. Another potential combination is the dual blockade of HER2 and SRC which was recently shown to work as a central node downstream of multiple trastuzumab resistance mechanisms [108]. Finally, HER3 is another strong activator of PI3K/AKT signaling pathway that has been demonstrated to be upregulated after HER2 blockade [109]. Although still in early phases of development, Rb disruption strategies and the use of promising molecules such as CDK-4/6 inhibitors may open new perspectives in the future [110].

Conclusion

Anti-HER2 treatments represent one of the most dynamic evolving fields for oncologists, as a recent great expansion in the drugs developed to target HER2-positive breast cancer raised new challenges.

Nevertheless, treatment personalization according to specific disease or patients' characteristics remains a matter of research.

Among the aspects to be improved and still under investigation, there is how to spare some side effects as cardiotoxicity. Serum cardiac biomarkers, including troponins and natriuretic peptides, represent possible tools to detect cardiotoxicity at a preclinical level and may represent an important means of selecting treatment to avoid side effects [111].

Another aspect is the possibility to de-escalate treatment for low-risk, early-stage, HER2-positive disease. The excellent disease-free survival observed with adjuvant paclitaxel and trastuzumab without anthracyclines has set a new standard of care for the adjuvant treatment of small ($T < 3$ cm), node-negative, HER2-positive breast cancer.

Recently ZEPHIR trial revealed through PET-CT assessment that almost half of the patients with advanced-stage HER2-positive breast cancer had substantial heterogeneity in HER2 expression between different metastases [112]. Pretreatment imaging of HER2 targeting, combined with early metabolic response assessment, holds great promise for improving the understanding of tumor heterogeneity in metastatic breast cancer and for selecting patients who will/will not benefit from HER2-targeted therapy.

Finally, our understanding about the mechanisms of trastuzumab resistance is still limited. The search for biomarkers to predict response and resistance is a critical part of ongoing research. Some studies suggest that the definition of HER2-driven cancers should be expanded to include both rare cases with somatic HER2-activating mutations (without gene amplification) [113] and HER2 positivity defined by gene expression.

The recognition of specific molecular predictors of response to emerging therapies will allow a more personalized approach to the treatment of HER2-amplified breast cancer. Enrolling patients into clinical trials, with the purpose to understand and target the molecular mechanisms involved in HER2 therapy resistance, is of crucial importance. As even more potential therapies appear over the horizon, advancements in biomarker discovery will be critical in optimizing treatment selection and providing personalized therapy for patients.

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Adjuvant Treatment with Bone-Targeting Agents (Bisphosphonates and Anti-RANK-Ligand Antibody)

48

Michael Gnant

Bone health and breast cancer are connected subjects that make bone health a particularly important subject for breast cancer patients: First, breast cancer patients have a higher prevalence of osteopenia and reduced bone health than non-breast-cancer women of the same age [1]. While the underlying biological reasons for this finding are not well understood, patients' bone health is further impaired by most of today's state-of-the-art therapies: Most endocrine interventions reduce estrogen levels and, subsequently, lead to rapid bone loss in both premenopausal and postmenopausal women [2]. Also, cytotoxic chemotherapy may exert detrimental effects on bone health: Chemotherapy leads to ovarian dysfunction in many premenopausal women, and some cytostatic drugs have direct nonhormonal effects on the bone [3, 4].

Aromatase inhibitors (AI) have become a standard treatment for endocrine responsive breast cancer of postmenopausal patients. It has been shown in several large trials of both steroidal and nonsteroidal aromatase inhibitors that these agents lead to significant bone loss and can increase the incidence of fractures [5, 6]. Postmenopausal women receiving an aromatase inhibitor will on average lose approximately 2–3% of their bone mineral density (BMD) per year while on treatment, or more than twice the typical rate for "physiological" bone loss during menopause [5].

In premenopausal women, cancer treatment-induced bone loss may be even more dramatic because of the profound and rapid nonphysiological reduction in peripheral estradiol achieved by current anticancer therapies: Ovarian failure as a result of chemotherapy occurs in 40–90% of all premenopausal women, depending on age [7], and is accompanied by a rapid decrease in BMD (up to 7% decrease within 12 months) [2]. In premenopausal patients receiving endocrine combination therapy, bone loss is similarly dramatic [8].

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Many experts believe that the prevalence of fractures caused by treatment-induced bone loss is severely underreported in pivotal AI trials, and some real-life reports of clinical practice show alarming figures [9]. In the ABCSG-18 trial [10], with its primary focus on bone health, the recorded rate of fractures in the placebo group (estimated 10% at 3 years, 16% at 5 years, and 26% at 7 years) notably exceeds previous reports from large adjuvant aromatase inhibitor trials [11].

Overall, bone health can thus be regarded as the Achilles heel of modern adjuvant anticancer treatment approaches in breast cancer. Pharmacologic interventions may help to prevent bone loss and subsequent fractures and preserve patients' quality of life. Bone-targeted agents have thus become an integral component of care for patients with postmenopausal breast cancer [12, 13]. Many guidelines and recommendations nowadays request that breast cancer patients on endocrine therapy should be monitored for bone loss and antiresorptive intervention considered when bone mineral density decreases. However, the value of routine DXA scans can be questioned since this established and rather inexpensive method may underestimate the true magnitude of the deleterious impact of endocrine therapy impairing bone health [14]. Furthermore, patient's and family history as well as lifestyle factors may likely add to an individual patient's fracture risk, as assessed by the popular FRAX score [15].

Based on the results of clinical trials, both oral bisphosphonates such as clodronate and intravenous aminobisphosphonates such as zoledronic acid are used to prevent and treat treatment-induced bone loss [16, 17]. However, recommendations vary as to which patient actually needs antiresorptive therapy from the beginning of endocrine therapy. Moreover, bisphosphonate use has been limited by side effects (compliance with oral bisphosphonates is low because of gastrointestinal sequelae), and caution is advised with intravenous bisphosphonates with respect to renal safety and dental problems. In addition, while adjuvant bisphosphonates usually stabilize bone mineral density, their effect on fracture prevention is less well defined.

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This has changed with ABCSG-18 [10]: In this randomized, placebo-controlled, double-blind trial of over 3400 postmenopausal breast cancer patients, the anti-RANK-ligand antibody denosumab dramatically decreased the incidence of clinical fractures (HR = 0.5, $p < 0.0001$). These pivotal results of adjuvant denosumab (at the "bone-protecting" dose of 60 mg twice a year) follow previous demonstrations of fracture prevention by denosumab in the noncancer osteoporosis setting [18] and the demonstration of bone mineral density stabilization in a phase II trial in breast cancer patients [19]. Interestingly, ABCSG-18 showed that breast cancer patients with apparently normal bone at baseline derived similar benefit from adjuvant denosumab as those who were already osteopenic at the time of diagnosis.

Bone-targeted therapies have also been investigated in the adjuvant breast cancer setting because of their antitumor properties: After early reports that adjuvant clodronate would improve breast cancer outcomes [20, 21], there were several large studies undertaken to investigate this fascinating subject (Table 48.1). Several of them (ABCSG 12, Z/ZO-FAST, AZURE) showed improved adjuvant outcomes when zoledronic acid was added to the standard adjuvant therapy. The ABCSG-12 trial showed a 29% risk improvement for disease-free survival (DFS) in premenopausal patients [22].

From this trial, long-term follow-up results at 84 months in patients receiving 3 years of therapy with goserelin and tamoxifen or anastrozole with or without ZOL ($N = 1803$) are available [23]: Patients receiving ZOL had significant reductions in the risks of DFS events and death versus the no-ZOL group ($p \leq 0.01$ for both), supporting the potential for carryover anticancer benefit with ZOL. Adverse events that were increased in patients receiving ZOL compared to without ZOL were related to the acute-phase reaction after ZOL infusion (arthralgia, bone pain, and pyrexia). No cases of renal failure or ONJ were reported in this study. Multivariate analyses revealed a strong interaction between ZOL and patient age. Subgroup analyses by age showed that the DFS and survival benefits observed in the overall patient population were mostly derived from patients over 40 years of age (risk of DFS events =0.66, $p = 0.013$; risk of death =0.57, $p = 0.042$) rather than younger patients ($p > 0.05$ for both). Patients over 40 years of age may have achieved more complete ovarian suppression during therapy; therefore, these results are consistent with data from the postmenopausal setting. Thus, current evidence supports the addition of ZOL to standard adjuvant therapy in premenopausal patients with hormone-sensitive BC undergoing ovarian suppression. However, factors that influence hormonal suppression remain unclear.

A retrospective analysis of ABCSG-12 showed that body mass index (BMI) may influence hormonal suppression and/or aromatase availability during adjuvant therapy. Patients with a BMI ≥ 25 kg/m^2 and treated with anastrozole had an increased risk of disease recurrence (60%; $p = 0.02$) and death (twofold; $p = 0.01$) versus patients with a BMI < 25 kg/m^2 [24]. In addition, overweight patients treated with anastrozole had worse outcomes compared with tamoxifen. However, estrogen levels were not obtained in this study, so the contribution of hormonal suppression versus aromatase availability could not be exactly elucidated.

Table 48.1 Summary of large randomized bisphosphonate trials

AZURE	Placebo-controlled phase III study evaluating the benefit of ZOL in patients with early stage BC	No OS differences in overall population; however, subset analysis in patients showed that: • Among post-menopausal patients, the 5-year rate of invasive DFS was 78.2% in the ZOL group and 71.0% in the control group (HR = 0.75; $P = 0.02$) • Among patients who had undergone menopause >5 years before study entry, the 5-year OS rate was 84.6% in the ZOL group versus 78.7% in the control group (HR = 0.74; $P = 0.04$)
ABCSG-12	Placebo-controlled phase III study evaluating the benefit of ZOL in pre-menopausal patients with early stage BC	DFS benefits observed at 48-month follow-up (HR = 0.74; log-rank $P = 0.01$) were maintained at 84 months (HR = 0.71; log-rank $P = 0.011$). Subset analyses at the 84-month follow-up show that DFS benefits appear to be driven by patients >40 years of age
ZO-FAST	Immediate versus delayed ZA plus adjuvant letrozole	Reduction in DFS (HR = 0.59) at 36 and 48 months. Disease recurrence reduced at bone and at nonbone sites
Z-FAST	Immediate versus delayed ZA	Decreased recurrence at 12–48 months, not at 60 months
NSABP–B34	Placebo-controlled phase III Oral clodronate stratified by HR and nodal status, and by age <50 or ≥50 years	In patients ≥50 years of age, clodronate improved: • RFI: HR = 0.76; $P = 0.05$ • BMFI: HR = 0.61; $P = 0.024$ • nBMFI: HR = 0.63; $P = 0.015$ • OS: HR = 0.80; $P = 0.1$
GAIN	Randomized controlled, 2 × 2 factorial design trial daily ibandronate or observation	No difference in the 3-year DFS (HR = 0.945; $P = 0.59$) or the 3-year OS (HR = 1.04; $P = 0.80$) between ibandronate versus observation in the ITT population

In postmenopausal patients, recent long-term results from large phase III studies of the intravenous bisphosphonate zoledronic acid support the potential for clinical benefits from intravenous bisphosphonates in postmenopausal women. Three similarly designed studies (Zometa-Femara Adjuvant Synergy Trials: Z-FAST, ZO-FAST, and E-ZO-FAST) enrolled postmenopausal women only and examined the effects of immediate versus delayed initiation of ZOL on disease recurrence and/or DFS as secondary endpoints. The final 5-year results of Z-FAST reported a lower incidence of disease recurrence in the immediate-ZOL groups (5.3% versus 7.0% for the delayed-ZOL group), with reductions in all types of distant recurrence (not only skeletal recurrence). The safety profiles were similar between the immediate-ZOL and delayed-ZOL groups, with no confirmed cases of ONJ and any grade of renal impairment reported in 2% and 1.3% of patients, respectively.

Similar efficacy results were observed at 5 years in the immediate- versus delayed-ZOL groups of ZO-FAST (local recurrence, 5 versus 12 patients, respectively; distant recurrence, 29 versus 41 patients, respectively). Consistent with the reduced incidence of disease recurrence, there was an overall 34% relative risk reduction for DFS events at 5 years' follow-up in the immediate-ZOL group versus the delayed-ZOL group ($p = 0.0375$). Reported adverse events were consistent with the known safety profiles of letrozole and zoledronic acid, with no increases in renal adverse events in ZOL-treated patients. Three confirmed cases of ONJ were reported in patients receiving the bisphosphonate. Patients who were truly menopausal (defined as naturally occurring menopause before study entry) experienced the majority of DFS events (92 events). However, the 29% relative risk reduction in truly menopausal immediate-ZOL-treated patients did not achieve statistical significance, possibly because the analysis lacked statistical power. An analysis of postmenopausal patients in ZO-FAST using the definition from previous studies (menopause >5 years before study entry or >60 years of age) showed a 37% relative risk reduction for the immediate-ZOL-treated patients ($p = 0.052$). Similar trends were obtained for OS in the two postmenopausal groups, with a significant OS improvement with immediate ZOL in established postmenopausal women (HR = 0.50; $p = 0.02$). Notably, among the patients in the delayed-ZOL group in ZO-FAST, 27% initiated ZOL for postbaseline fractures or BMD decreases (median time, 12.8 months), further confounding accurate assessment of the DFS benefits with ZOL. Indeed, exploratory analyses revealed that initiating ZOL was the only factor to influence DFS events in the delayed-ZOL arm (HR = 0.462; $p = 0.033$) [25]. The E-ZO-FAST had shorter follow-up and very few DFS events.

In the overall "negative" AZURE study [26], the only predefined variable to affect disease recurrence, invasive DFS, and OS with ZOL versus control was menopausal status. Postmenopausal women constituted 45% of the study population, and in patients with established menopause (entering menopause ≥5 years before study entry; 31% of the overall trial population), adding ZOL to standard therapy improved invasive DFS (HR = 0.75; $p = 0.02$) and OS (HR = 0.74; $p = 0.04$). These patients are obviously expected to have the lowest hormone levels regardless of anticancer regimens used, which strongly suggests that the anticancer potential of aminobisphosphonates is influenced by the prevailing hormonal milieu rather than by the concomitant systemic therapy or age in women with breast cancer. The results of the AZURE study also support earlier evidence from ABCSG-12 and ZO-FAST suggesting a systemic anticancer effect of zoledronic acid in and outside of the bone. The incidence of serious adverse events was similar between the ZOL and placebo groups; ONJ incidence was low and as expected for these treatment regimens (1.1% versus 0%, respectively). The adverse events were not stratified by age or menopausal status, which precludes knowing if there was a bias toward postmenopausal women.

The GAIN study investigated adjuvant ibandronate, and while the study overall did not show a DFS difference (as AZURE), there was a positive trend with respect to DFS in postmenopausal trial patients [27]. Similarly, there was a significant difference in the subgroup of patients older than 50 years of age in NSABP-34, whereas the overall trial did not show an outcome benefit for 3 years of oral clodronate [28]: In the postmenopausal subgroup, which comprised 64% of the overall trial, a small, nonsignificant improvement in DFS was seen with clodronate. However, recurrence-free, bone metastasis-free, and distant metastasis-free intervals were all significantly improved by the addition of clodronate to standard care in postmenopausal women ($p < 0.05$ for all). A post hoc analysis of skeletal metastasis development showed that the benefit from clodronate was driven by patients who were 60 years of age or older at study entry. This further supports the existing evidence that patients with the lowest hormone levels derive the greatest anticancer benefits from bisphosphonate therapy in the adjuvant setting. However, in this trial 44% of patients who initially received clodronate did not complete treatment (3-year follow-up), and whether oral bisphosphonates can really provide clinical benefits to this patient population in a real-world setting remains a bit uncertain at this time.

Interestingly, all these large trials demonstrate a clear-cut pattern of dependence of a beneficial effect of bisphosphonates on breast cancer outcomes on menopausal status [29]. The "metastasis-preventing" effect of bisphosphonates was seen in

Table 48.2 DFS effects of bisphosphonates in postmenopausal trial subgroups

Study	"Postmenopausal" DFS (95% CI)	*P* Value
AZURE (n = 1041)[a]	0.75 (0.59–0.96)	0.02
ABCSG XII (n = 1390)[b]	0.66 (0.48–0.92)	0.013
ZO-FAST (n = 1065)[c]	0.66 (0.44–0.97)	0.04
NSABP-B34 (n = 2139)[d]	0.68 (0.5–0.92)	0.013
CLODROPLAC (n = 539)[e]	0.66 (0.49–0.93)	0.007
GAIN (n = 1557)[f]	0.75 (0.49–1.14)	0.17

DFS in postmenopausal subsets of large adjuvant bisphosphonate trials

[a]Coleman RE, et al. N Engl J Med. 2011;365:1396–1405
[b]Gnant M, et al. SABCS 2011. Abstract S1-2
[c]De Boer R, et al. SABCS 2011. Abstract S1-3
[d]Paterson A, et al. SABCS 2011. Abstract S2-3
[e]Powles T, et al. Breast Cancer Res. 2006;8:R13
[f]Mobus V, et al. SABCS 2011. Abstract S2-4

postmenopausal women (and in premenopausal women who were rendered postmenopausal by receiving ovarian function suppression) (Table 48.2) but not in premenopausal patients where DFS was not improved by adjuvant bisphosphonate therapy [30]. Despite some experimental data exist that propose scientific explanations for the differential impact of silencing the microenvironment in differing menopausal states [31], this correlation is not perfectly understood.

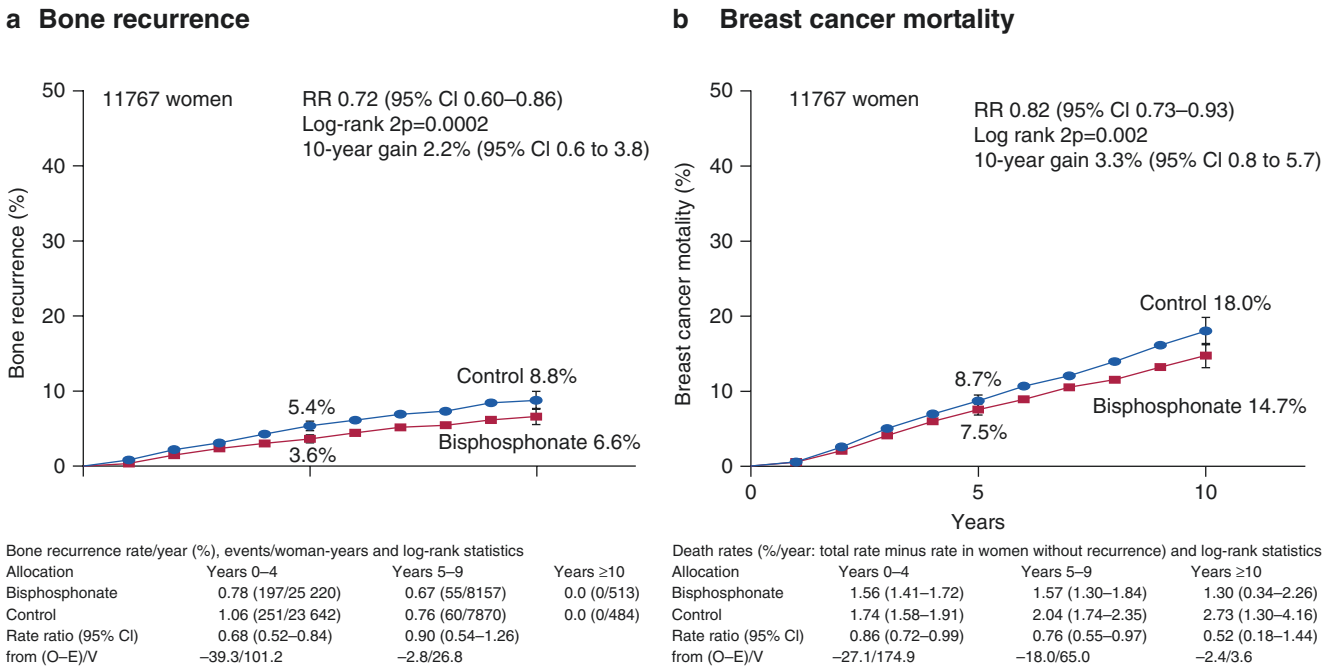
Some of these trial results were discussed in the scientific community in a truly controversial manner and sparked a discussion about the actual putative underlying mechanism of the antitumor effects of adjuvant bone-targeted therapies [32]. While "direct" anticancer properties have been well described in experimental settings (sometimes at bisphosphonate doses that cannot be achieved in the clinical setting), it appears more likely that an indirect effect on the bone marrow microenvironment is actually the foundation for the observed outcome benefits in the adjuvant setting [33, 34]. In fact, the idea that the microenvironment plays an important role in oncology has been proposed already more than a century ago by Sir Stephen Paget ("seed and soil" theory) [35]. However, this is not generally accepted, and understandably so since there are also indications for a "direct" antitumor activity, both experimentally and in the neoadjuvant clinical setting [36, 37]. Another issue that remains unclear is whether bone-targeted therapies actually can prevent metastasis outside the bone in the adjuvant setting, for which there are some indications also in the clinical setting, but most trials show primarily a "bone-only" benefit [38].

In terms of clinical consequences, the debate was eventually settled by the global meta-analysis of all adjuvant bisphosphonate trials, done by the Bisphosphonate Working Group of the Early Breast Cancer Trialists' Collaborative Group based in Oxford, UK. In this worldwide collaborative process involving over 20,000 patients from most of the randomized bisphosphonate trials ever performed, a small but significant benefit for adjuvant bisphosphonates in terms of DFS as well as overall survival was shown for postmenopausal breast cancer patients [39]. Interestingly, the meta-analysis did not indicate a difference between oral and intravenous bisphosphonates, but the overall relative reduction of breast cancer recurrences in postmenopausal patients was a relative 14% (HR = 0.86, p = 0.002), translating into absolute differences of 2.4% at 5 years and 3.0% at 10 years of follow-up (Fig. 48.1).

In summary of these results, adjuvant effects of bisphosphonates are now scientifically established not just as treatment aiming at the reduction of bone loss, but also yielding a decrease (prevention) of bone metastasis at least in postmenopausal patients [40], in some trials even a reduction of recurrences outside the bone [41]. However, lack of approved market access indications in most healthcare environments remain a major obstacle in making this treatment available for the majority of breast cancer patients.

Furthermore, ABCSG-18 fracture data—while demonstrating a dramatic reduction in fractures (HR = 0.5, $p < 0.0001$)—further added to some confusion of the clinical routine implications when they were first presented at ASCO 2015 [10]: Would the bone-health "aware" physician now have to recommend denosumab for the prevention of fractures but (and?) bisphosphonates for the prevention of recurrence [42]? Fortunately, an IDMC-recommended early DFS analysis of ABCSG-18 [43] presented at San Antonio in December 2015 indicates that adjuvant denosumab reduces recurrences in a similar manner than bisphosphonates do in postmenopausal patients (HR = 0.82, p = 0.05). This DFS benefit translates into a 1.2% absolute DFS benefit at 3 years, 2.1% at 5 years, and 3.1% at 7 years of follow-up. In higher-risk subgroups, the absolute benefit may be numerically larger, e.g., tumor size >2 cm: 3.7% at 3 years, 7% at 5 years, and 10.5% at 7 years, respectively.

In summary, there is no doubt that bone-targeted therapy plays an important role for the adjuvant therapy of postmenopausal breast cancer patients (or premenopausal patients on ovarian function suppression therapy): Adjuvant bisphosphonates both protect bone mineral density and reduce recurrences [44] and are therefore recommended for clinical practice despite regulatory and market access limitations [45]. The most recent results of ABCSG demonstrate that adjuvant denosumab similarly stabilizes bone mineral density and reduces disease recurrence, but in addition cuts treatment-induced (clinical = relevant!) fractures in half [10, 43]. With this innovative treatment addition that cuts fractures in half and improves disease-free survival similarly to what bisphosphonates do in postmenopausal women, and importantly without measurable toxicity at the low dose of 60 mg s.c. twice yearly, patients can derive considerable benefit at a burden that can be considered minimal.



Bone recurrence rate/year (%), events/woman-years and log-rank statistics

Allocation	Years 0–4	Years 5–9	Years ≥10
Bisphosphonate	0.78 (197/25 220)	0.67 (55/8157)	0.0 (0/513)
Control	1.06 (251/23 642)	0.76 (60/7870)	0.0 (0/484)
Rate ratio (95% CI)	0.68 (0.52–0.84)	0.90 (0.54–1.26)	
from (O–E)/V	–39.3/101.2	–2.8/26.8	

Death rates (%/year: total rate minus rate in women without recurrence) and log-rank statistics

Allocation	Years 0–4	Years 5–9	Years ≥10
Bisphosphonate	1.56 (1.41–1.72)	1.57 (1.30–1.84)	1.30 (0.34–2.26)
Control	1.74 (1.58–1.91)	2.04 (1.74–2.35)	2.73 (1.30–4.16)
Rate ratio (95% CI)	0.86 (0.72–0.99)	0.76 (0.55–0.97)	0.52 (0.18–1.44)
from (O–E)/V	–27.1/174.9	–18.0/65.0	–2.4/3.6

Fig. 48.1 (Adapted from [40]) Bone recurrence (**a**) and breast cancer mortality (**b**) in postmenopausal women from the EBCTCG's overview

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Systemic Treatment for Specific Medical Situations

49

Silvia Dellapasqua

In the last few centuries, medicine progresses have ensured an increasingly longer life expectancy and have allowed people to live with chronic diseases which were lethal in the past. As a consequence, the proportion of patients of all ages living with chronic comorbid conditions, and in particular of elderly patients, is rapidly increasing. Aging remains one of the single greatest risk factors for the development of new breast cancers. Approximately 50% of breast carcinomas occur in women ≥65 years, and more than 30% of breast carcinomas occur among women >70 years [1]. Older women represent the fastest growing segment of the population in the United States and in Europe [2]; therefore, during the coming decades, older women will represent an increased cohort of patients with newly diagnosed disease and survivors [3, 4]. In addition, recent advances in the field of oncology have also contributed to significantly improve disease outcomes for most type of cancers. As a consequence, oncologists have often to face issues related to breast cancer occurring in other malignancy survivors.

49.1 Aging and Assessment of Older Patients

Aging is characterized by a progressive decline in the functional reserve of multiple organ systems, an increase in the prevalence of functional dependence, comorbidity, and memory disorders and a decline in economic resources and social support [5, 6]. These changes influence treatment-related decision-making for older individuals because they imply a decrease in life expectancy and tolerance of cancer treatment. These factors are poorly reflected in chronological age alone and should be considered when determining optimal treatment approaches for this age-group [7].

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A multidimensional comprehensive geriatric assessment (CGA) provides the most reliable information regarding life expectancy, treatment tolerance, social support requirements, and unsuspected conditions (e.g., dementia, depression, comorbidity) that may interfere with cancer treatment [8]. The CGA includes evaluation of functional status, comorbidity and pharmacy, socioeconomic conditions, cognitive status, emotional status, nutrition, and geriatric syndromes. The assessment of the functional status, other than with performance status, is defined as the measurement of a patient's ability to complete functional tasks, which range from simple self-care in activities of daily living (ADL, which includes feeding, grooming, transferring, and toileting) [9] to more complex instrumental activities of daily living (IADL, which includes shopping, managing finances, housekeeping, laundry, meal preparation, ability to use transportation and communicating by telephone, and the ability to take medications) [10]. Comorbidity is assessed by the number and the severity of comorbid conditions (comorbidity index), and pharmacy is assessed by evaluating the number of medications, appropriateness of medications, and risk of drug interactions. Socioeconomic conditions are assessed by evaluating the living conditions and especially the presence and adequacy of a caregiver. The cognitive status is evaluated through the Mini-Mental State Examination by Folstein and other tests, whereas the emotional status is evaluated through the Geriatric Depression Scale (GDS). Nutrition is assessed through the Mini-Nutritional Assessment (MNA). Geriatric syndromes are dementia, delirium, depression, falls, neglect and abuse, and spontaneous bone fractures.

Several studies have supported the effectiveness of CGA in improving functional status, reducing hospitalization, decreasing medical costs, and prolonging survival. In the older cancer patient, the CGA allows a gross estimate of life expectancy and of the functional reserve and tolerance of chemotherapy, the recognition of reversible comorbid conditions and special economic needs that may interfere with cancer treatment, and the management of nutrition and medications [11]. The CGA allows categorization into three stages of aging:

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- "Fit" patients lack severe comorbidities, are functionally independent and are candidates for any form of standard cancer treatment.
- "Frail" patients have dependence in one or more activities of daily living, three or more comorbid conditions and/or one or more geriatric syndromes, and are candidate only for palliative treatment.
- Patients with intermediate characteristics are defined as vulnerable and may benefit from some special pharmacological approach, such as reduction in the initial dose of chemotherapy with subsequent dose escalations [12].

All patients aged 70 and older should be subjected to some form of CGA because the prevalence of age-related problems increases after this age [13, 14]. Since the CGA is a time-consuming approach, at least an abbreviated screening version should be performed [13, 15] with the full assessment being given only to those patients who screen positive in some domains.

49.2 Frailty

A key point in the patient evaluation in the onco-geriatric setting is the definition and identification of frailty, which includes being dependent on others, being at substantial risk of dependency and other adverse health outcomes, experiencing the loss of "physiological reserves," having many chronic illnesses, having complex medical and psychosocial problems, and having "atypical" disease presentations [11]. Current criteria for the recognition of frailty include age over 85 years, dependence in one or more activities of daily living, three or more comorbid conditions, and the presence of one or more geriatric syndromes [16].

Frailty is a reversible condition characterized by a high degree of susceptibility to external changes that require adaptation and compensation. When cancer is the external change, the main objective of frailty detection is to adopt compensatory strategies acting at different levels [11].

49.3 Biology of Breast Cancer in the Elderly

In general, breast cancer appears more indolent with increasing age [17–19]. Approximately, 70–80% of all breast cancers occurring in elderly patients express estrogen receptors, tend to grow slowly, are usually better differentiated, respond to hormonal treatments, and are associated with a longer disease-free interval and a slightly better overall prognosis [23]. There is, however, a 20–30% of patients who remain at high risk of relapse because of extensive nodal involvement or estrogen receptor-negative disease.

49.4 General Principles of Cancer Treatment in the Elderly

Although available clinical data demonstrate that treatment efficacy is not modified by age, elderly patients are underrepresented in clinical trials [20, 21]. In a review of SWOG trials, only 9% of elderly patients with breast cancer were entered into trials, despite 49% of all elderly cancer patients having breast cancer [22]. Likewise, in the Oxford Overview of 60 trials involving 29,000 women comparing chemotherapy with none, only 4% were 70 or older [23]. Older patients are more likely to have conditions that make them ineligible for clinical trials because of protocol exclusions mainly related to comorbidities or ageist trial designing [24]. Consequently, to date, most data concerning older women with breast cancer are derived from retrospective studies, which are often affected by selection bias [23].

The challenge of caring for older women is tailoring treatment to fit the patient. Although this is true for all patients, it is mostly relevant in elders, in whom comorbidity and functional loss can lead to undertreatment and shorter breast cancer-specific survival or to overtreatment and toxicity [25]. Older women with breast cancer are often affected by age-related comorbidities, which may limit treatment options [26]. Older women are less likely to receive treatment in concordance with guidelines, including surgery, radiotherapy, chemotherapy, and endocrine therapy, regardless of their breast cancer stage [27].

49.5 Adjuvant Treatment

49.5.1 Endocrine Treatment

Adjuvant endocrine therapy should be recommended to women whose breast cancer contains hormone receptors, regardless of age, menopausal status, involvement of axillary lymph nodes, or tumor size [28]. Tamoxifen has long been the most commonly used hormonal treatment, with data supporting a 5-year course rather than shorter periods [23]. However, only 1 year of treatment had a significant effect on disease-free and overall survival up to 21 years in elderly patients as shown by data from the International Breast Cancer Study Group (IBCSG) Trial IV [29].

Aromatase inhibitors have shown to reduce breast recurrence as compared to tamoxifen in a number of trials. Two analyses have been done specifically in elderly patients. In the MA.17 trial, the advantage conferred by extended letrozole after 5 years of tamoxifen was significant only in patients younger than 60 years. However, since there was no significant interaction between age and treatment for disease-free

survival or overall survival, extended adjuvant therapy with letrozole could be considered for healthy elderly patients [30]. In the Breast International Group (BIG) Trial 1-98, letrozole showed age-independent superior efficacy compared with tamoxifen [31].

In older patients, aromatase inhibitors are preferred to tamoxifen because of the lower risk of increased thrombosis and endometrial cancer, with similar effect on quality of life [30, 31]. However, aromatase inhibitors are associated with musculoskeletal syndrome, accelerated bone loss and increased fracture rate irrespective of age, as suggested prospectively in BIG 1-98. BIG 1-98 results showed significantly more grade 3–5 protocol-specified non-fracture adverse events for letrozole compared with tamoxifen in patients ≥75 years, whereas differences were not significant for thromboembolic or cardiac events [31]. Cognitive impairment has been described in association with adjuvant hormonal treatment, but data are sparse [32]. Bone loss associated with aromatase inhibitors is a particular problem in elderly patients, since preexisting decreases in bone mineral density and osteoporosis are prevalent. Vitamin D and calcium supplementation should be considered, especially since subclinical vitamin D insufficiency is common in elderly patients. Antiresorptive therapies are indicated for increasing bone mineral density and reducing fracture risk in elderly patients with osteoporosis [33].

In a recently published update of the meta-analysis by the Early Breast Cancer Trialists' Collaborative Group, aromatase inhibitors reduced recurrence rates by about 30% compared with tamoxifen. Moreover, 5 years of an aromatase inhibitor reduced 10-year breast cancer mortality rates by about 15% compared with 5 years of tamoxifen, hence by about 40% compared with no endocrine treatment [34].

In older patients with small hormone receptor-positive tumors, endocrine therapy with either aromatase inhibitors or tamoxifen is the mainstay of treatment [35]. However, the optimal way of using aromatase inhibitors or tamoxifen as endocrine treatment for early breast cancer remains uncertain. The American Society of Clinical Oncology Technology Assessment published recommendations for adjuvant treatment that included aromatase inhibitors, either upfront or as a sequential therapy consisting of tamoxifen followed by aromatase inhibitors in the treatment of postmenopausal women with endocrine responsive breast cancer [36]. In general, aromatase inhibitors may be preferable as initial treatment in most elderly patients because, unlike tamoxifen, they are not associated with endometrial carcinoma and do not increase the need for yearly gynecologic examinations in older women who have not had a hysterectomy. Although arthralgia and myalgia are less frequent in older patients treated with aromatase inhibitors, they can result in pain and functional loss and create a cause for discontinuing therapy [35].

49.5.2 Chemotherapy

Several randomized trials have shown that chemotherapy improves both disease-free and overall survival in women with early breast cancer, but a lesser absolute benefit from chemotherapy has been observed with increasing age. In fact, the proportional reduction in risk of recurrence and mortality seems to decrease with increasing age; however only 4% of patients included in the overview analysis were >70 years [23]. The optimal chemotherapy regimen, doses, and schedules for the adjuvant treatment of elderly patients have not yet been defined, while concern is increasing regarding the toxicity associated with chemotherapy in this patient population [37].

Only recently, specific trials addressing adjuvant chemotherapy in older women have been conducted, and their results are available. In the French Adjuvant Study Group 08 (FASG 08) trial, fit elderly women aged ≥65 years, with node-positive early breast cancer, were randomized to tamoxifen with or without weekly epirubicin. The 6-year disease-free survival showed a nonstatistically significant improvement (72.6% vs. 69.3% $p = 0.14$) in favor of combination arm; the relative risk of relapse in multivariate analysis was significantly higher in patients who received tamoxifen alone, compared with patients treated in combination (HR 1.93, $p = 0.005$) [38]. The IBCSG CASA trial evaluated pegylated liposomal doxorubicin and low-dose, metronomic cyclophosphamide and methotrexate in women ≥66 years with estrogen and progesterone receptor-negative breast cancer. After 2 years, the trial closed early, due to slow accrual. At a median follow-up of 42 months, 81% of patients were free of breast cancer recurrence [39]. The CALGB trial evaluated a standard regimen (AC or CMF) as compared with capecitabine in patients older than 65 years and showed the superiority of a standard adjuvant chemotherapy in women with hormone receptor-negative tumors [45]. The ICE trial completed the accrual of 1409 patients older than 64 years of age with node-positive or high-risk node-negative early-stage breast cancer who were deemed inappropriate for conventional treatment and who were randomized to ibandronate or the combination of ibandronate and six cycles of capecitabine. Results showed no difference between the two treatment arms for the primary endpoint of 3-year invasive disease-free survival and overall survival [40]. The ICE II trial randomized 400 women ≥65 years with high-risk breast cancer to standard arm (four cycles of EC or six cycles of CMF) vs. experimental arm (six cycles of weekly nab-paclitaxel plus capecitabine (PX)). Interim safety analysis revealed that EC or CMF were more tolerable than PX. The rates of invasive disease-free survival were equivalent between the two arms (HR 0.98, $p = 0.9597$) at 48 months [41]. The ELDA Trial randomized 302 patients with

node-positive or high-risk node-negative tumors to classical CMF or weekly docetaxel. At a 70 months median follow-up, weekly docetaxel did not show to be more effective than standard CMF. Hematological toxicity, mucositis, and nausea were worse with CMF; allergy, fatigue, hair loss, onychopathy, dysgeusia, diarrhea, abdominal pain, neuropathy, cardiac, and skin toxicity were worse with docetaxel. Quality of life was worse with docetaxel for nausea/vomiting, appetite loss, diarrhea, body image, future perspective, treatment side effects, and hair loss items [42]. The Weekly nab-paclitaxel (Abraxane®) Versus Epirubicin (WAVE) trial is an ongoing phase II trial evaluating activity and quality of life of women with early breast cancer who are elderly or unfit for a 3-week polychemotherapy regimen and who are randomized to either weekly epirubicin or weekly nab-paclitaxel both for 16 weeks.

The decision to recommend chemotherapy to an older patient with early-stage breast cancer is complicated and requires knowledge of life expectancy, the risks and benefits of the proposed treatment, and the patient's and family's goals for treatment [43]. In general, for healthy older patients with estimated survivals of 10 years or more, state-of-the-art treatments similar to those used for younger patients should be recommended. For patients with an average life expectancy of less than 5 years, the value of adjuvant endocrine therapy and certainly chemotherapy is likely to be minimal except in the case of patients with extremely high-risk disease [35].

In older patients with hormone receptor-positive, HER2-negative tumors, the absolute benefit in improving survival with the addition of chemotherapy to endocrine therapy is highly dependent on the risk of tumor recurrence. In general, the majority of patients with node-negative tumors will derive little benefit from chemotherapy. Patients with four or more nodes should receive chemotherapy if their life expectancy exceeds 5 years. Patients with a life expectancy of less than 5 years, irrespective of nodal involvement, are not likely to derive any benefit from chemotherapy [35].

For older patients with triple-negative breast cancer and a life expectancy exceeding 5 years, the major systemic treatment consideration is chemotherapy. Several chemotherapy regimens are appropriate in these patients [44]. In general, regimens such as doxorubicin and cyclophosphamide [45] or docetaxel and cyclophosphamide [46] are preferred. However, anthracyclines are associated with increased risks of cardiac toxicity and the development of acute myelogenous leukemia and myelodysplasia, whereas taxanes have substantial risks of peripheral neuropathy, a potential toxicity that can impede function and impair the quality of life of older patients [47]. It is essential to inform patients and families of potential major toxicities since for a few percent gain in survival, many older patients might decline chemotherapy if it is likely to affect their physical function [35].

49.5.3 Trastuzumab

Limited data are available on adjuvant trastuzumab in elderly patients. The combined NSABP B-31/NCCTG N9831 US trials of standard chemotherapy with or without adjuvant trastuzumab for 1 year involved 16% patients over 60 years old, and their benefit with trastuzumab was at least as good as for younger women (HR 0.51) [48]. Likewise, in an exploratory subgroup analysis of the HERA trial which also had 16% of patients over the age of 60, there was no significant difference in disease-free survival benefit in the older group compared with younger women [49]. However, the standard chemotherapy used in these trials is likely to be too toxic for many elderly or frail patients. Recently a large phase II study investigated adjuvant weekly paclitaxel for 12 weeks with trastuzumab in 406 "low-risk" patients with <3 cm, node-negative HER2-positive breast cancer, including 24% of patients aged 60–69 and a further 10% aged >70 (34% >60). This study showed outstanding disease control with an estimated relapse-free survival of 98% at 3 years [50].

A systematic review of prospective randomized trials in patients >60 years showed a significant 47% relative risk reduction in patients receiving trastuzumab compared to chemotherapy alone, with a 5% pooled proportion of cardiac events [51].

Older patients with HER2-positive tumors can benefit from chemotherapy and trastuzumab, the greatest absolute benefit being observed in patients with hormone receptor-negative, HER2-positive tumors [35]. The combination of weekly paclitaxel and trastuzumab is a reasonable option for elderly or frail patients with node-negative HER2-positive breast cancer [50]. More aggressive regimens should be considered for older patients with higher-risk breast cancer [52].

A major concern when offering chemotherapy and trastuzumab to older patients is the risk for cardiac toxicity [53], which is enhanced in anthracycline regimens. Before the initiation of chemotherapy, patients should have an estimation of left ventricular ejection fractions (LVEF) using either echocardiographic or nuclear medicine methods.

49.6 Neoadjuvant Treatment

Patients with locally advanced disease might be offered preoperative systemic therapy to render surgery feasible or to make breast conservation possible.

Most elderly patients have ER-positive HER2-negative breast cancers, for which neoadjuvant endocrine therapy can be extremely effective, possibly as effective as chemotherapy [54].

A Cochrane review showed a decrease in local progression with surgery plus endocrine treatment compared with primary endocrine therapy alone; however, no difference

was observed in overall survival. For optimal local control, surgery (with or without radiotherapy) plus adjuvant endocrine therapy is better than primary endocrine therapy. Evidence exists for disease control of 2–3 years with primary endocrine therapy [55]. Therefore, in patients with a short life expectancy (<2 years), considered unfit for surgery or refusing surgery, primary endocrine therapy might be considered.

Primary endocrine therapy studies have mainly used tamoxifen, although aromatase inhibitors could be preferable on the basis of neoadjuvant, adjuvant, and metastatic data [56–58]. The ESTEEM trial comparing primary anastrozole with surgery plus adjuvant anastrozole in women ≥75 years was closed because of low accrual. Clinical trials of primary aromatase inhibitors in frail older patients with ER-positive tumors are needed, but in view of the difficulty in recruiting for such a trial, it is reasonable to assess each individual for tamoxifen or aromatase inhibitors based on potential toxicity.

For patients with triple-negative breast cancer and good life expectancy, anthracycline and taxane regimens can be used [35]; however, no specific data is available in older patients.

For patients with HER2-positive disease, neoadjuvant therapy that includes pertuzumab in addition to trastuzumab provides the best chances for tumor reduction [59, 60]; however, specific data is lacking in older patients.

49.7 Metastatic Breast Cancer Treatment

Endocrine treatment is the treatment of choice for older women with ER-positive metastatic breast cancer [61]. Chemotherapy is indicated in older patients with ER-negative, hormone-refractory, or rapidly progressing disease. Elderly patients with metastatic breast cancer are expected to derive similar benefits from chemotherapy as younger patients. Single-agent chemotherapy is generally preferred to combination regimens, which are usually more toxic and provide, at most, a limited survival gain. Preference should be given to chemotherapy agents with better safety profiles (such as weekly taxanes, pegylated liposomal doxorubicin, capecitabine, and vinorelbine) that have been studied in older patients [62]. There is limited data on polychemotherapy in elderly patients. Combination oral chemotherapy (vinorelbine and capecitabine) was assessed in patients >70 years with advanced cancer, many with breast cancer, and was active and well tolerated [63]. Oral therapy is attractive since it eliminates the constraints and risks of parenteral therapy, but efficacy and tolerability can be compromised by interference with food (e.g., lapatinib), concomitant medications (e.g., capecitabine with warfarin), and errors in compliance. Dose reductions and schedule modifications are controversial but should be considered based on pharmacology and toxicity [61].

Patients with HER2-positive disease should receive HER2-targeted therapy and chemotherapy. In elderly patients with HER2-positive, ER-positive disease with a contraindication to chemotherapy, or without life-threatening disease, anti-HER2 therapy plus endocrine therapy is an option. In elderly patients with HER2-positive, ER-negative disease who are unfit for chemotherapy and without life-threatening disease, trastuzumab monotherapy could be reasonable. However, there are no specific efficacy or safety data in elderly patients. First-line trastuzumab monotherapy showed clinical benefit rates of around 40% [64]. Combination anti-HER2 plus hormone therapy (trastuzumab plus anastrozole, lapatinib plus letrozole) improves progression-free survival over hormone therapy alone in ER-positive, HER2-positive disease, but with more toxic effects and higher economic cost [65, 66]. Trastuzumab and lapatinib are equally effective in younger and older patients with metastatic breast cancer [61]. Data on trastuzumab in elderly women are limited, but a retrospective series showed that benefits and safety seem to be conserved in patients older than 60 years and in those older than 70 years [67]. Lapatinib plus capecitabine has similar efficacy in older and younger women [68]; however, elderly patients are less tolerant of diarrhea-associated dehydration and need close monitoring [69].

Bisphosphonates and denosumab are underused in elderly patients [33, 70]. Special considerations should be made for elderly patients, who might have renal impairment or might be taking concomitant medications for comorbid conditions. In this regard, there could be an advantage for denosumab in elderly patients. Because of noncompliance with oral bisphosphonates, intravenous or subcutaneous administration might be preferable [61].

49.8 Management of Frail Patients

With the expansion of the older population, the number of frail elderly and frail elderly with cancer is expected to rise. Approximately 400,000 frail elderly in the United States are affected by some form of cancer at any given time [16, 71]. Management of cancer in the frail person is mainly comprised of palliation. The use of opioids is complicated by delirium, constipation, and nausea, and these side effects may become so disturbing that an older patient may prefer to tolerate pain rather than the symptoms related to pain management [72]. However, recent drug developments offer options to the frail cancer patients. In addition to the use of bisphosphonates for bone metastases, new antitumor agents including capecitabine, low weekly doses of taxanes, liposomal doxorubicin, vinorelbine, and gemcitabine may be beneficial to these individuals while producing minimal toxicity.

49.9 Systemic Treatment for Other Malignancy Survivors

Cancer survivors can be affected by a number of health problems, but often their greatest concern is facing cancer again. In fact, certain types of cancer and cancer treatments can be linked to a higher risk of certain second cancers.

The most common second cancer seen in survivors of breast cancer is another breast cancer [73]. For some second cancers, shared genetic risk factors may play a role. For example, women with mutations in the BRCA genes have an increased risk of both ovarian cancer and breast cancer, as well as of other tumors [74]. Another risk factor shared by both breast cancer and other malignancies is aging. Moreover, prior cancer treatments, including radiation, chemotherapy, and certain drugs, also have risks associated with developing secondary and unrelated cancers. Finally, lifestyle (including diet, exercise, smoking, UV exposure, and alcohol intake) can have an impact on incidence of breast cancer as well as of other tumors. Women who have had breast cancer can get any type of second cancer, but they have an increased risk of ovarian, endometrial, thyroid, stomach, colorectal and lung cancer, melanoma of the skin, soft tissue sarcomas, and acute myeloid leukemia [75].

Patients surviving after a first tumor diagnosis and newly diagnosed with breast cancer present some treatment issues, mostly related to cumulative toxicities of treatments.

The most commonly associated toxicities from chemotherapy occur in tissues composed of rapidly dividing cells and may spontaneously reverse with minimal long-term toxicity. However, myocardium consists of cells that have limited regenerative capability, which may render the heart susceptible to permanent or transient adverse effects from chemotherapeutic agents. Such toxicity encompasses a heterogeneous group of disorders, ranging from relatively benign arrhythmias to potentially lethal conditions such as myocardial ischemia/infarction and cardiomyopathy [76]. For this reason, caution should be exerted in patients who received prior anthracyclines for other neoplasms, since their cumulative dose (450 mg/m^2 for doxorubicin and 900 mg/m^2 for epirubicin) should not be exceeded [77].

Neuropathy induced by chemotherapy is an increasingly frequent problem, for which neither prophylaxis nor specific treatment is available, and only symptomatic treatment can be offered. The most frequent chemotherapeutic drugs causing peripheral neuropathy are platin compounds, vinca alkaloids, taxanes, bortezomib, and thalidomide. The role of synergistic neurotoxicity caused by previously given chemotherapies and concomitant chemotherapies and the role of preexistent neuropathy on the development of peripheral neuropathy is not clear. As the number of long-term cancer survivors increases and a new focus on long-term effects of chemotherapy-induced neuropathies emerge, rehabilitation needs to be implemented to improve the patients' functions and quality of life [78].

Radiation and adjuvant chemotherapy for breast cancer are associated with a risk of myelodysplastic syndrome and acute myelogenous leukemia, which continues to increase beyond 5 years [79]. This risk is particularly evident with the use of alkylating agents, in particular cyclophosphamide, which is commonly used in breast cancer treatment. In order to minimize the risk of marrow neoplasms, cyclophosphamide cumulative dose should not exceed 36 g [80].

Tamoxifen increases the risk of endometrial cancer [81]. Women taking tamoxifen should be informed about the risks of endometrial proliferation, endometrial hyperplasia, endometrial cancer, and uterine sarcomas, and any abnormal vaginal bleeding, bloody vaginal discharge, staining, or spotting should be investigated. In a patient with prior history of endometrial tumor, tamoxifen is contraindicated, and other endocrine treatments should be prescribed instead.

Radiation therapy to the breast increases the risk of lung cancer, esophageal cancer, and sarcomas. The risk increases over time and is highest 15 or more years after breast cancer diagnosis [82]. Conversely, even 40 years after treatment, survivors of Hodgkin's lymphoma (HL) are at increased risk for breast cancer, which accounts for more than 40% of the excess risk of a second cancer among women with previous HL. As compared with mantle-field irradiation, radiation therapy with less-extensive supradiaphragmatic fields was associated with a substantially lower risk of breast cancer [83]. In patients with prior mantle-field irradiation for HL and newly diagnosed with breast cancer, re-irradiation of the breast can lead to tissue necrosis. For this reason, mastectomy remains the standard of care in most cases [84].

Conclusions

In elderly women with breast cancer, in addition to the existing evidence from clinical trials and retrospective studies, practitioners need to take into consideration the functional status, social support, patient preference, presence of comorbidities, and life expectancy when selecting optimal treatment, weighing the risks and benefits of all therapeutic options. There is no absolute age limit for the use of standard chemotherapy regimens. Rather, the use of such treatments should depend on disease characteristics, comorbidity, life expectancy, and patient preference. In general, standard treatments should be offered to "fit" elderly patients irrespective of age. However, standard adjuvant chemotherapy can be difficult for elderly or frail patients and is associated with an increased risk of serious morbidity and treatment-related mortality. Simple well-tolerated, short-duration, single-agent schedules may be preferable for elderly and/or frail patients who may prefer

to trade a very minor reduction in efficacy for a much easier treatment. There is an urgent need for further clinical trials of less toxic chemotherapy schedules which may be as effective as standard with very little trade-off in efficacy.

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Part VIII

Radiotherapy

Conceptual Basis and Principles of Radiation Oncology

50

Roberto Orecchia

Radiation oncology is a wide discipline of human sciences that joints many of the conceptual basis of physics, biology, and medicine and is funded on the knowledge of the fundamental principles of (1) cancer and normal tissue molecular biology, (2) basic and medical physics and dosimetry, (3) physical and biologic interaction of radiation with normal and malignant tissues, (4) high-precision imaging, and (5) the effect of the combined use of radiations with other treatment modalities, such as surgery, drugs, and other physical energies. The multidisciplinary education of radiation oncologists is the foundation for a high quality of the patient's care.

50.1 Aim of Radiation Therapy

Radiation therapy is the clinical modality that transfers concepts and principles of radiation oncology in the clinical setting. Radiation therapy is a locoregional treatment suitable for the treatment of cancer and occasionally some benign diseases. To realize this goal, radiation therapy uses different sources of ionizing radiation. The most important characteristic of ionizing radiation is the localized release of a large amount of energy in the human tissues. This energy is able to break the chemical bonds of the atoms or molecules and initiate the chain of events that lead ultimately to a biologic effect, fundamentally the DNA damage.

Radiation therapy allows to deliver a precisely measured dose of irradiation to a defined volume of clinical interest, with the minimal damage as possible to the surrounding healthy tissue or organs. The goal is eradication of the disease (care), possibly with preservation of organ functions and form, and therefore, maintaining a high patient's quality of life, and, last by not least, at competitive cost when compared with other therapies [1]. Radiation therapy is also used for pain treatment and other symptoms relief, with the aim to perform palliative care in incurred disease, maintaining the patients comfort but also often prolonging their life [2].

Today, over 50% of patients with cancer will receive this treatment at some times during the management of their malignant disease, and radiation therapy is an integral part of the management of the most frequent cancers worldwide, including breast, lung, prostate, head and neck, and cervix [3]. In spite of the well-recognized role, radiation therapy is often absent from global health plans and receives limited funding [4]. As a result, there is a worldwide shortfall of radiation therapy services, especially in low-income countries, where more than 90% of the population lacking access to radiation therapy. But even in high-income countries, radiation therapy has frequently been used in suboptimal way, despite facilities being easily available. The growing incidence of cancer will require to increase the capacity of the radiation therapy services. In 2012, worldwide, more than 14 million new cases of cancer were reported, and this number is projected to reach about 25 million by 2030 [5]. In 2012, five cancers, lung, breast, colorectal, prostate, and stomach, comprised almost half the total incidence of cancer and caused more than 50% of the eight million cancer deaths. The incidence and profile of cancer vary between and within the different countries [6]. Country-specific variability in cancer incidence, as estimated in 184 countries worldwide, shows breast cancer as the leading cause of cancer in 73 countries, including parts of Central and South America, Africa, and Asia. Prostate cancer is the most frequent cancer in 34 countries, mostly those with high life expectancy and diffuse testing for PSA (Americas, northern and western Europe, Oceania). Cervical cancer is most common in 26 countries, mainly in low-income countries, as sub-Saharan Africa and in parts of South America. Lung cancer is the

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most frequent cancer in 18 countries, including parts of eastern Europe, western Asia, northern Africa, and eastern Asia. Colorectal cancer incidence is highest in an almost equivalent number of countries, mainly in Europe and in eastern Asia. Radiotherapy is needed to treat most of these cancers as part of a course of evidenced-based, effective care.

In Europe, about four million new cancer patients are predicted in 2025, representing a 16% increase in the absolute number of cancer with respect to 3.4 million diagnosed in 2012 [7]. Also in Europe, the number of expected cases is not uniformly distributed across the different countries. This situation will require to treat by radiation therapy about two million of patients in 2025, with a majority of them having breast, lung, prostate, and head and neck cancers.

To treat cancer, radiation therapy can be used as sole treatment modality, to permanently eradicate the primary tumor and regional node metastasis, or in combination with surgery, both preoperatively, with the aim to inactivate a large proportion of tumor clonogenic cells and shrink inoperable or borderline operable tumors, or postoperatively, to eliminate residual subclinical cancer deposits on the tumor bed or positive margins remained in the tissues surrounding the resected area. The preoperative irradiation of a undisturbed area allows the use of smaller radiation portals and lower radiation dose than that recommended postoperatively. On the other side, a potential advantage of the postoperative approach is that the extent of the gross disease and microscopic margins is well defined, and radiation therapy can be specifically tailored to the involved sites. Regardless of whether radiation therapy is given before or after surgery, the time interval between the treatments is important to reduce the possibility of tumor cell repopulation. To overcome this problem and reduce the risk of any delay in the combined approach, intraoperative irradiation can be also considered, with the use of a high single dose at the time of surgery, directly at the tumor bed [8].

In locally advanced stages of cancer, radiation therapy is also frequently used in combination with chemotherapy. Induction or neo-adjuvant chemotherapy can be given weeks or months before radiation therapy to reduce the tumor volume and improve the global effectiveness of the treatment. Concurrent or concomitant chemotherapy is given during the course of radiation in a wide range of indications to enhance, as a sensitizer, the radiation effects on tumor. The optimal schedule of administration depends on the particular drug, and the benefit occurs only when the cancer cell killing effect is greater than the expected increase of toxicity on normal tissue. Adjuvant chemotherapy is used to eradicate occult distant cancer spread after the completion of radiation therapy and the achievement of local and regional control of the tumor. More recently, advances in the knowledge of molecular radiation biology are providing the rationale for combining radiation with new targeted drugs able to modulate signaling pathways, opening investigations on feasible and promising novel therapies [9].

50.2 Sources of Radiation

Radiation therapy has been used for more than 110 years for the treatment of patients [10]. Shortly after the discovery of X-rays in 1895, both low-energy X-rays and radium sources were used in Europe and America, and due to the poor penetration of these sources, mostly for the treatment of superficial tumors. During the early 1900s, early experiments in radiobiology were conducted, in parallel with the development of new machines. Clinical experience with these units suggested advantages of high-energy radiation (megavoltage) over the available low-energy generators (kilovoltage) at that time. High-energy radiotherapy delivered by ^{60}Co (cobalt) machines was developed in the 1950s and allowed to deliver the dose at depth, even with relatively limited penetration. The photons, with two picks of energy, respectively, at 1.17 and 1.33 MeV, are produced from the radioactive decay of the source. The intensity of the radiation decreases with time, and the ^{60}Co source must be changed every 5 years to avoid too long treatment time.

Such considerations have led centers to replace cobalt units with modern linear accelerators. These machines, developed since the 1960s, are multimodality and provide a wide range of photon and electron energies, from 4 MV up to 25 MV, with the guaranty of much greater penetration in the most deep-seated tumors and smaller penumbra. Clinically, 4–8 MV beams are the most useful providing a good balance between penetration and surface dose. In an era of personalized medicine, technical progress means that linear accelerators have been implemented with sophisticated tools, such as multileaf collimators, allowing to shape and modulate beams to conform to the exact shape of tumors, maximizing radiation dose deposition in the cancer, while sparing normal tissues from high doses, those most likely to evoke toxic effects.

One strategy to further improve the precision of radiation therapy, focusing more the dose in the target volume, is the use of heavy charged particles. This radiation modality is also named hadrontherapy. The most employed particle in clinical setting is protons. Protons have a mass of 2000 times that of the electrons and require to be accelerated at the therapeutic range of energy (up to 250 MeV) by very huge special machines, as cyclotron or synchrotron. Protons have the same biological effectiveness of photons. Other heavier particles, as carbon ion, produce more dense ionization and increase of three to four times the damage to the DNA of the cancer cells [11].

In addition to external beam sources, brachytherapy has been used since the early 1900s, placing radium sources.

Radium therapy was developed for about 40 years, mainly in Paris and Manchester. New sources were introduced after the Second World War, with iridium-192 and caesium-137. Exposure of the staff became a top priority, and in the following years, technological improvements allowed to realize the modern approach, with the placement of nonradioactive source carriers before, with more time to their accurate positioning, followed by the radioactive loading after. Today, brachytherapy is realized by sophisticated machines that allow the mobilization of sources from a protected storage safe via flexible transit tubes to various types of treatment applicators (after/remote loading). These techniques have expanded the scope and the range of applications of brachytherapy [12].

50.3 Radiation Therapy Planning

Planning is a critical step in the delivery of clinical radiation therapy. Treatment to be effective has to be delivered to the region of interest, using different techniques and regimens. Delivery of radiation therapy requires firstly the accurate definition and delineation of volume to be treated, into a precise anatomic context [13].

Conventionally, different sub-volumes have been identified. The GTV (gross tumor volume) is corresponding to the macroscopic feature and location of the disease, as shown by imaging. The GTV may be different when it is determined by different imaging modalities or when, using the same modality, different image viewing parameters are used. The CTV (clinical target volume) includes the GTV and the regional area at risk for the spread of the disease (subclinical disease) and constitutes the volume to be irradiated with an adequate dose to control the tumor. In case of previous resection of the tumor mass, it's possible to have a CTV without a GTV. The PTV (planning target volume) takes into account uncertainties related to the treatment, including patient's movement and variation in patient setup. The equally critical identification is reserved to identify organs at risk (OaRs), surrounding the tumor area. OaRs are by definition site-specific and require to be analyzed on individuals in terms of dose constraints, volume, and risk factors [14].

In the daily routine practice, CT scan is used to identify these volumes. CT images closely simulate the effects of radiation passing through the patient, being the CT voxel value, which reflects X-ray attenuation, depending on the electron density of the body tissues. Current technology of CT images, by spiral or helical acquisition, strongly reduces the time with the advantage of minimizing the magnitude of organ motion during the examination but sometimes also introducing motion artifacts. When appropriate, MR and PET images can be also imported into the planning to provide more sophisticated information, such as tumor metabolic activity [15].

Planning is strongly depending on complex computer algorithms to calculate the dose distribution due to the beam passing though the human body, including the altered electron density across different tissues. This pretreatment process has to be implemented on a daily basis on the linear accelerator, to guarantee accuracy and reproducibility. The importance of immobilization device, setup control, and electronic portal imaging is well recognized, in the frame of strict quality assurance programs. Currently, linear accelerators can be equipped with imaging modalities that allow the acquisition of anatomical images of the patient in treatment position or during the treatment. For the radiation source, megavoltage (MV) treatment beam or kilovoltage (kV) X-ray source can be employed; both can provide bi- or three-dimensional (cone beam CT) images.

Strategies to verify target shape, volume, and position and to correct the topographical inconsistencies with the original plan are part of image-guided radiotherapy (IGRT). IGRT uses, in addition to the previously mentioned tools, also other various devices, such as real-time ultrasound, optical tracking imaging, and fiducial markers placed on the body surface or implanted within the target (clips). In the case of substantial deviations from the original treatment plan that cannot be adjusted by means of couch, machine, or MLC shifts, it becomes necessary to replan and re-optimize the dose distribution. This kind of adaptive radiotherapy can be done offline, with a time lapse, or even online, with a fast replanning system to elaborate a new plan for the current treatment session. Besides inter-fraction motion, also intra-fraction motion has become relevant because of the relatively long time to deliver the dose. To overcome this problem, treatment rooms can be equipped with movement tracking systems to monitor and compensate for target motion during irradiation, due to uncontrolled physiological behavior, such as coughing or body relaxation. The MR linear accelerator (MR-linac) represents a further step toward a fully adaptive intra-fraction planning system. More precise tracking of target and organs at risk, thanks to advanced soft tissue visualization, should lead to narrower safety margins around the target and to smaller treated volumes, making it possible to further escalate the dose safely [16].

Radiation therapy is administered to the patients according to different regimens. The most used is named as "conventional fractionation," and according the current practice in America and Europe, this regimen is delivered in a large number of fractions, from 30 to 40, over 6 and 8 weeks, with a rest during the weekend. For most cancers, the curative treatment provides a fractional dose of 1.8–2.0 Gy, given once a day. The total dose is determined by the type and the size of the tumor and by the tolerance of critical structures encompassed in the target volume. Usually, the total dose is in the range of 70–80 Gy when the entire tumor mass is present and must be eradicated, and 50–65 Gy when the treatment is given to control the subclinical disease, after or

before surgery, or in the area at risk. Lower dose is prescribed in case of very sensitive tumors to radiation, such as seminoma, lymphomas, and other hematological malignancies.

There are many other different regimens, considered "altered fractionation," some of them based on empiric observations. In "hyperfractionation," the total dose is slightly increased, the size of the dose per fraction is significantly reduced, the number of fractions is increased, and the overall time is substantially unchanged, due to the possibility to give two fractions per day, with an interval between the two of at least 6 h. In "accelerated fractionaction," the overall time and number of fractions are reduced, and the dose per fraction increased, remaining the total dose either unchanged or somewhat reduced, depending on the extent of the overall time reduction. One of the most frequent applications of the concept of accelerated schedule is the use of the boost dose (and additional dose given to the tumor bed or to the mass) concomitantly to the normal schedule. Concomitant boost allows to reduce of 1 or 2 weeks the total time, increasing the dose per fraction on only a part of the treated volume. In "hypofractionation," the overall time and the number of fractions are strongly reduced, in some case at only one or few sessions. The higher dose per fraction can be a disadvantage for the increased risk in the severity of late response in normal tissue but also an advantage when this regimen is applied in palliative treatment or in highly precise techniques, such as stereotactic radiosurgery.

50.4 Clinical Radiation Biology

Radiation biology studies the sequence of events following the absorption of energy from ionizing radiation, the response of the normal and cancer cells and tissues to compensate, and the damage that may be produced [17]. When a radiation penetrates and releases its energy into a biologic tissue, a complex series of actions start. There are two possibilities of interaction, the direct or indirect ionization. The indirect mechanism is predominant with photons, as X-rays or gamma rays, that are considered sparsely ionizing radiations. Photons give up their energy in various interactions and then utilized to produce fast-moving electrons as secondary particles that interact with the most abundant cellular medium, water. The radiolysis (splitting) of the water involves a series of reactions that produces free radicals, causing, in their turn, reactions with normal component of the cells and target molecules, including DNA. The direct ionization is caused by charged particles (protons, ions, or electrons) as a result of the incident particle itself because of the relatively densely ionizing nature of most particulate radiations. When a particle causes ionization, it loses energy and may give off most of its energy just prior to stopping. This effect is referred as the Bragg peak.

The amount of energy deposited into the tissue is a function of distance along the track of the radiation. Considering this aspect, radiations are also divided according to different linear energy transfers (LET), and the amount of energy deposited in a unit of track, into high LET (densely ionizing, as particles) and low LET (sparsely ionizing, as photons). The value of LET is strongly correlated with the biologic effect of radiation (RBE—relative biological effectiveness). More energy is transferred to the tissue and higher is the entity of the damage. The RBE can vary slightly from tissue to tissue, and also when acute and delayed effects of radiations are compared, but in general, is assumed that RBE is equal to 1.0 for photons, 1.1 for protons, 3.0 for carbon ions, and up 5.0 for neutrons.

As previously described, exposure to ionizing radiations creates lesions within cellular DNA that cause a range of responses, including cell cycle arrest, apoptosis, reproductive death, and senescence. The consequences of these types of damage are quite different. Loss of a DNA base changes the sequence and causes alterations on protein synthesis, including mutations if the genetic material is involved. In case of single-strand break (SSB), and not repaired, a major damage can occur. In this plethora of lesions, there is evidence that the DNA DSB (double-strand break) is the most important in terms of tumor and normal tissue radiosensitivity. About 40 DSBs are induced in a cell for each Gy of absorbed dose, and these lesions are responsible for radiation-induced cell death, with a single unrepaired DSB sufficient to elicit this response. Several features of DSBs are more difficult to be repaired compared with single-strand breaks and other forms of genetic damage. The response to different types of DNA damage involves the recruitment of proteins needed for DNA repair with overlapping functions and other proteins with functions that are specific to a particular lesion or process. These proteins can also regulate, through either apoptosis or mitotic death, the subsequent fate of the cell or its progeny.

Abnormalities in the response have consequences for cellular radiation radiosensitivity as measured by clonal death and for spontaneous and radiation-induced genomic instability. This is the reason because cells and tissues can markedly vary in their expressed sensitivity. Generally, rapidly dividing cells that are poorly differentiated and with a long mitotic period are very sensitive, not dividing or slowly dividing cells are less radiosensitive or more radioresistant. Typical examples of very or radiosensitive cells are lymphocytes, spermatogonia, ovarian follicular cells, and cells of the intestinal epithelium, head and neck and gastric mucosa, and others. An intermediate grade of radiosensivity is manifested in fibroblasts and cells of the glandular epithelium of the breast and epithelium of other tissues, as pulmonary, renal, pancreatic, and thyroid. Radioresistant cells are mature hematopoietic, connective, bone and cartilage, and muscle and ganglion

cells. Because of the presence of a strong heterogeneity of cell lines, the radiosensivitity of a given organ can vary according to the mixture of various components.

The radiobiology of breast cancer is not easy to determine, because the main role of radiotherapy is the eradication of residual subclinical disease after mastectomy or breast conservation surgery, and tumor response cannot be directly observed. Also outside the patient, breast cancer has been difficult to study because of its resistance to growth in cell culture. Therefore, radiobiological data have been mostly derived from clinical studies. With respect to the dose–response relationship, the common standard is represented by 50 Gy in 25 fractions (conventional fractionation) to the whole breast/thoracic wall and, if indicated, to the regional lymph nodes. In case of breast-conserving surgery, or positive margins after mastectomy, an additional dose (boost) is often suggested, in the range of 10–16 Gy in 2 Gy fractions.

A remarkable set of data on the relationship between total dose, fraction size, and local control are available. The data were analyzed using the linear quadratic model, a nonconceptual model, but a robust empirical model of fractionation sensitivity. In this model, there are two assumed components of radiation damage characterized by the coefficients α and β. The α component results from a single ionizing event that simultaneously damages two individual targets. This damage cannot be repaired, and increases with dose, in linear pattern, and is influenced more by overall dose and not by fraction size. The β component is resulting from two ionizing events which separately damage two targets. These targets are sublethally damaged, and only the combination of them can form a lethal lesion. The β damage increases with the square of the instantaneous dose and is influenced by both overall dose and fraction size. The α/β ratio is a measure of how a tissue will respond to a change in total dose or fractionation (fraction sensivity). For early-reacting (days or weeks after radiation) normal tissues and tumors the α/β value is high, of 10 Gy or more. With late-responding (years after radiation) normal tissues, the α/β value is low, of 5 Gy or less. The value estimate for human breast cancer is in the range of 4–5 Gy. At this stage, it looks likely that breast cancer shares, on average, a similar sensitivity to fraction size as late-responding normal tissue.

On the basis of these data, to intercompare different regimens of radiation therapy, the biologically effective dose (BED) is a useful linear quadratic-based parameter. Equivalence in BED is the base to perform randomized clinical trial among different whole-breast radiation therapy dose schedules. Currently, a parallel standard of fractionation has been identified, in 40 Gy in 15 fractions or 42.5 Gy in 16 fractions, both over 3 weeks. Radiation therapy confined to the tumor bed (partial breast irradiation) in women with low-risk tumors treated by complete excision is an emerging strategy in several countries, but none of the current studies are designed specifically to test the importance of treatment time. In these schedules, the treatment times are highly compressed to one, with a dose of 20–21 Gy, to five fractions of 5.7–6.0 Gy each. There is a strong impact of the α/β value in the equivalence of BED. For example, assuming a value of 10 Gy, 21 Gy in single dose is equivalent to 56 Gy in conventional fractionation, but if we consider, as estimated breast α/β value, the value of 3.5–4 Gy, the BED increase up to more than 100 Gy. A comprehensive review of clinical tolerance and dose–effect correlations of the most commonly irradiated organs was organized in the Quantitative Analysis of Normal Tissue Effect in the Clinic (QUANTEC) project [18]. Models linking dose with toxicity and tumor control, based on radiobiological and mathematical principles, were used to predict the normal tissue complication probability (NTCP) and tumor control probability (TCP), enabling radiation oncologists to evaluate the potential treatment outcome [19].

50.5 Techniques for Radiation Therapy in the Breast

The efficacy of radiation therapy in increasing local and locoregional control and reducing breast cancer and overall mortalities is well established. From the point of view of radiation techniques, standard and new approaches were developed along the decades [20]. Today, modern techniques offer the ability to incorporate improved target imaging, accurate planning, and high-precision treatment delivery into the treatment design, also ensuring the possibility to use safely different schedules of fractionation [21].

In breast radiation therapy, the patients are traditionally placed in supine position, flat on the couch, or, more frequently, on an angled board with one or both arms stretched above the head. The advantage of the angled board is that the sternum can be brought horizontal, reducing angulation in the beam geometry. Other more sophisticated immobilization devices can be also used, to further reduce the setup uncertainties and limiting the intra-fractional motion. As example, patients can be positioned in a vacuum bag system, with their arms extended over their heads and holding a T-bar. The vacuum bag forms a template on which the patient can lie, with a high reproducibility of the position from treatment to treatment. Recently, prone positioning of patients was suggested for better sparing of normal organs, particularly in patients with large or pendulous breasts. Lung doses could significantly be reduced, whereas the sparing effect on the heart was less clear, because of anterior displacement of the heart in this position. Furthermore, the risk of acute skin reactions is reduced, because of the elimination of skin folds. The effect of this position on breathing motion is positive, with a smaller intra-fraction motion. On the other side,

patient setup variations can be wider, leading to an increased inter-fraction change. Dedicated breast boards were developed to establish a stable prone patient position with the breast hanging free from the thoracic wall, away from the target organs. Dose homogeneity can be improved by the prone technique, with some uncertainties at the medial and lateral borders of the breast, where the target coverage may be impaired, due to the limited accessibility for treatment fields in this position.

In the 2D technique, a standard field setup is used, with two opposed tangential fields at 180°, covering the breast, and field edges based on surface anatomy. To treat supraclavicular and axillary nodes, an anterior or anterior oblique field is used. The lower border of this field is matched to the upper border of the tangential beams. To treat mammary internal chain, a direct mixed photons and electron beams can be used or, as an alternative, modified wide tangent technique. For treatment planning, only a limited amount of contours of the patients' outline are available, with limited account for individual variations in patient anatomy. Today, 2D dose distribution with the standard technique is difficulty acceptable when the target volume becomes more complex and aberrations in dose homogeneity occur. Wedges are used for missing tissue compensation, but can only adjust for dose heterogeneity in a two dimensional plane, and optimal dosimetry is not achieved for a significant number of patients. Furthermore, the inclusion of the locoregional lymph nodes in the target volume makes treatment planning more difficult, due the high inhomogeneity of the dose distribution at the match lines of the different fields.

Currently, the challenge is to minimize normal tissue complications, improve cosmetic outcome, and reduce the overall treatment time without losing treatment efficacy. Technological advances allowed the development of new techniques that are able to improve the radiation dose distribution and dose delivery.

The definition of three-dimensional conformal radiation therapy (3D-CRT) concerns the geometric matching of the irradiated volume to the target to be treated. 3D-CRT allows the integration of CT scan information in the planning system, enabling the design of radiation fields that are based on patient-specific 3D anatomy. Special devices, such as multileaf collimators implemented in linear accelerators, can deliver these conformal beams to the patient. Individualization of treatment results in a more optimal dose distribution reducing inhomogeneity due to individual variability in the curvature of the chest wall, separation of the heart from the breast target, and size and shape of the remained breast tissue. The achievement of these goals can be assessed by inspection of the 3D dose distribution, by the interpretation of dose–volume histograms for the outlined structures, and by the calculation of the conformity index that exactly describes the fraction of the planned target volume covered by the treated volume. Using these tools, the amount of data to be evaluated when judging the adequacy of a treatment plan can be significantly reduced. In breast-only irradiation, the 3D alignment of the tangential beams allows improved shaping and coverage of the breast tissue and reduction of the volume of the irradiated heart and lung tissue. In locoregional breast irradiation, the problems of inhomogeneous dose distribution that can frequently occur at the match lines between the breast and lymph node fields can be solved with 3D techniques, by the use of asymmetric collimation or, using full CT scan data, the customization on the patients' anatomy. Comparisons between different locoregional 3D techniques show that every technique has advantages and disadvantages, and the decision which technique to use should be patient-specific, considering tumor-, patient-, and treatment-related characteristics, taking into account the quality and the attitude of the radiation therapy tools and team. For some patients in which the summation of the dose from the uniform selected beams is not acceptable, more advanced techniques such as intensity-modulated radiation therapy may be beneficial.

Intensity-modulated RT (IMRT) is a treatment technique that allows an optimal modulation of the intensity of the beams, ideally divided into the individual rays within each beam ("beamlets"). The control of the fluence of each beam improves dose distribution, enabling a real customized design of the target volumes. IMRT plan requires special calculation algorithms able to guarantee computer-aided optimization methods. The current standard for radiation treatment planning uses some form of the convolution algorithm to calculate photon beam dose. Still more accurate dose calculations can be achieved by using Monte Carlo models, which used more in research that in clinics because of the need for more computational power with this system. To compute and optimize the IMRT dose distribution, a process of inverse planning is required. In the conventional treatment plan, radiation is given with spatially multiple uniform fields, with an ultimate goal to give a uniform and conformal dose at a specified predefined target. In the inverse planning process, the beam intensities needed to deliver the dose distribution that would achieve the desired clinical objectives are first determined. In practice, the planner specifies the dose distribution within the patients, and an inverse planning algorithm computes the optimum beam modulation to produce that distribution. For example, the dose to organ at risk can be defined as less than some maximum allowable dose, incorporating these dose–volume constraints in the optimization scheme ("no more than 20% of the total lung volume may receive more than 20 Gy). IMRT is based on different types of delivery (step and shoot, sliding windows, volumetric arc therapy, and Tomotherapy®). The dose distribution can be painted around the target volume with a steep dose gradient. For IMRT, the dose distribution is characterized by a concavity at the edge of the higher doses that fits well with breast conformation and spares OARs. Several techniques for

IMRT in breast cancer have been developed, varying from fairly simple conventional tangents to complex setups with multiple fields. For the irradiation of the breast alone, all these techniques improved dose uniformity and reduced doses to the heart and lung. Since the multi-field solutions often suffer from increased low doses to the surrounding normal tissues, most investigators propose a technique using two conventional tangential fields. A large amount of the dose is given by parallel-opposed open beams; a part of the dose is given by several MLC-shaped segments, each delivering a small amount of monitor units ([10]). These segments are generated to compensate for missing tissue and block organs at risk. The IMRT technique allows the simultaneous delivery of different dose levels to different target volumes within a single treatment fraction: this approach is defined as the "simultaneous integrated boost technique" (SIB). The SIB technique is of particular interest because it can be used to yield higher doses to the critical area (boost volume) without increasing the overall treatment time after breast-conserving surgery. The application of IMRT in the treatment of locoregional BC is more challenging. Due to the complex target volume, more advanced IMRT techniques are proposed, using multiple beams and inverse planning. There is no agreement in the orientation and amount of beams among the different planning studies. Up to 11 beams, covering an arc of 180–360°, are advocated. In general, all techniques were able to improve the dose distributions, compared to non-intensity-modulated 3D-CRT plans. Some IMRT techniques are good at sparing one structure, whereas others are better at sparing other structures. Depending on the technique used, increased doses to the contralateral breast, contralateral lung, esophagus, thyroid, and humeral head are reported. The optimal geometric beam arrangement is determined by the anatomy of the patient, the location of the target structures, and the desire to minimize radiation dose to healthy tissues. Therefore, the choice of the best technique must be patient-specific. Due to the use of sharp dose gradients in IMRT, the effects of patient setup errors and breathing motion on the dose distributions are more important and must be taken into account, when evaluating the potential gains of IMRT over 3D-CRT. Furthermore, the benefits of IMRT must be weighed against the increase in overall low radiation dose. In multiple beam IMRT, more monitor units are needed to deliver the desired dose. This results in more leakage radiation and a higher total-body dose. IMRT is often associated with image guidance (IGRT) to measure and correct positional errors of radiation fields immediately prior and during treatment delivery.

In order to reduce the breathing motion, a respiratory gating technique can be associated with IMRT. In this technique, the radiation treatment is synchronized with the patients' individual breathing pattern. The radiation beam is turned on only during a prespecified phase of the respiratory cycle, thereby modifying the relative position of the target structures and normal organs in the radiation field. Gating for breast irradiation seems to be most favorably done in the inspiration phase. During the inspiration, the distance between the breast and the heart is enlarged and the lung density is reduced. To perform a gated treatment, dedicated devices are available that can record the patients' breathing pattern, allow for coaching of the patients to achieve the desired breathing pattern (deep inspiration breath hold or deeply free breathing), and gate the CT scan and treatment machine in the desired phase of the respiratory cycle. In breast locoregional treatment with wide tangential fields and IMRT compensation, the use of a moderate deep inspiration breath hold (mDIBH) technique can result in large benefit in reducing the dose at the heart.

Accelerated partial breast irradiation (APBI) is a technique designed to treat only the tissue surrounding the cavity after lumpectomy. A reduction in treatment volume allows for the delivery of larger treatment fractions in a shorter time period, usually from 1 to 10 days. A variety of treatment techniques are developed to deliver APBI. Interstitial multicatheter brachytherapy requires the highest level of skill but also offers the most flexible and adaptable approach. Multiple catheters are placed in the breast tissue surrounding the surgical cavity at 1–2 cm intervals. Interstitial brachytherapy can be administered with either a low-dose rate (LDR), a pulsed dose rate (PDR), or a high-dose rate (HDR) technique. Common dose delivery regimens for LDR and PDR are 45–50 Gy in 3–6 days and for HDR 32–34 Gy in 8–10 twice-daily fractions. Intracavitary balloon brachytherapy represents a simplification of multicatheter techniques. It relies on the placement of a radioactive source within a special balloon catheter device (Mammosite®) that fits inside the surgical cavity and treats 1 cm of tissue surrounding the cavity. MammoSite® employs only HDR regimens, with a typical fractionation of 34 Gy in ten fractions, twice daily. The major drawback to the use this technique is standard spherical dose distribution that can result in an overdosage to the skin. Therefore, it is mandatory to have a skin source distance of at least 10–15 mm. This limits the indication to deep-seated central surgical cavities in large breasts. More recently, other types of balloons were introduced, with multiple or stepping sources. They allow more flexibility in adapting the dose distribution to the shape of the cavity. In intraoperative radiation therapy (IORT), a single fractional dose (±20 Gy), targeted at the tumor bed, is delivered during surgery, using electrons in the energy range of 6–12 MeV or low energetic X-rays (50 kV). A major advantage of this approach is the complete skin sparing and the possibility to avoid the exposure of the underlying lungs and heart by shielding the thoracic wall with a lead/aluminum plate. A disadvantage is that radiation is completed before the final pathology report is known and that this information cannot be incorporated in the patient selection criteria. 3D-CRT

with and without intensity modulation is also used to perform APBI. Multiple beams are used to deliver 34–38 Gy in ten twice-daily fractions. Advantages of 3D-CRT/IMRT over the other APBI approaches include the non-invasiveness and the improved dose homogeneity with potential reduction in normal breast tissue toxicity. On the other hand, target volume definition and localization can be more difficult. Stereotactic radiation therapy (SRT) in breast cancer provides excellent results mainly in the treatment of metastases; it delivers a high dose/fraction for good local control, it exploits the steep dose gradient to improve tolerance, and it delivers the treatment in few fractions for an optimal quality of life. This technique can be also used in the treatment of primary tumor. The minimal treatment volumes and high-dose conformity are reminiscent of high-dose-rate interstitial brachytherapy without its known technical challenges and invasive requirements making CK-SAPBI quite appealing. Stereotactic APBI offers additional advantages of fewer treatments delivered and increased patient comfort due to lack of a second surgical procedure to place the brachytherapy applicator. Potential disadvantages are the fiducials required to track target motion. Fiducial migration can occur after placement, or fiducial tracking can be suboptimal in patients with poor breast integrity and large postoperative seromas. Some bioabsorbable tissue markers are now available in the attempt to overcome these limitations. Within the radiation landscape, proton therapy for APBI is still in its infancy, but the dose deposition characteristics of these particles make them very well suited for highly conformal treatments, with the potential for far less integral dose to the patient and greater avoidance of normal structures (heart, lung, and contralateral breast). Some preliminary reports seem promising, also in the case of extensive radiation fields.

50.6 Future Perspectives

In the radiotherapy setting, technological innovation has led to remarkable improvements in every phase related to treatment, from simulation to planning to delivery, with the aim of minimizing normal organ toxicity and improving local control [22]. The optimization of dose distribution limits the hot spots, areas receiving a higher dose than that prescribed, which could give rise to severe late effects. This phenomenon known as “double trouble” in conventional fractionation turns into “triple trouble” in the case of hypofractionation, where dose/fraction size is increased. Reducing exposure to the organs at risk (OARs) by means of precise 3D reconstruction decreases toxicity and paves the way to safe dose escalation. The combination of dosimetric data and data regarding clinical toxicity makes it possible to chart complex dose–response relationships and to define specific tolerance doses for OARs. Positive findings that reflect how technological advances translate into medical benefit are represented by the rise in local control, with its effect on survival, and by the ever lower cardiac toxicity. An essential aspect of the advances in physics and technology is expressed in the quality of treatment execution. High conformability means high sensitivity to any changes occurring in the patient during the course of radiotherapy. Displacement of the target due to organ motion, anatomical changes in the patient’s body, or inaccurate setup affects dose distribution, leading to inadequate target coverage or excessive irradiation of the OARs. This is of particular concern when dose escalation or hypofractionation is used. Moreover, innovative therapies, such as alternative radiation schedules or target agents, pose new challenges and increase the complexity. Biologic and molecular studies need for a better understanding of the phenomena related to tumor control and side effects.

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Whole-Breast Irradiation Following Breast-Conserving Surgery for Invasive Breast Cancer

51

Anna Kirby

51.1 The Rationale for Whole-Breast Irradiation

Irradiation of the whole breast in women who have undergone breast-conserving surgery (BCS) has long been a standard of care in the treatment of early breast cancer. This practice is predominantly based on the local control and survival gains demonstrated by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analyses [1, 2]. These are based on data from 10,801 women treated in 17 randomised trials of BCS plus or minus whole-breast irradiation (WBI) and demonstrate that the addition of WBI to BCS approximately halves the risk of local recurrence at 10 years (from 35% to 19%) and reduces the risk of breast cancer death at 15 years by around one sixth (from 25% to 21%). In women with pathologically node-negative disease (n = 7287), the risk of local recurrence was reduced from 31% to 16% at 10 years and the risk of breast cancer death from 21% to 17% at 15 years. In women with node-positive breast cancer (n = 1050), radiotherapy reduced the 10-year risk of local recurrence from 64% to 43% and the 15-year risk of breast cancer death from 51% to 43%. Consistent with previous meta-analyses, the prevention of four local recurrences at 10 years prevented one breast cancer death at 15 years.

In terms of risk factors for local relapse, on univariate analysis, the EBCTCG meta-analysis reports an association between the risk of local recurrence and young age, tumour size, grade and lymph node status [1]. Multivariate analysis of outcomes in a combined analysis of the EORTC 10801 and DBCG-82TM studies (both of which tested breast conservation surgery and whole-breast irradiation against radical mastectomy) also found an association between the risk of local relapse and young age and grade but not tumour size or lymph node status [3]. An additional association was found with the presence of lymphovascular invasion.

The EORTC boost trial demonstrated that addition of a boost dose to the tumour bed improved local control rates even further [4, 5]. Five thousand three hundred eighteen women were randomised to standard WBI to a dose of 50 Gy in 25 fractions plus or minus a boost to the tumour bed of 16 Gy in 8 fractions. Outcomes have now been reported at median follow-up of 17 years [5]. The addition of the boost dose reduced the 20-year risk of ipsilateral breast recurrence from 16% to 12% ($p < 0.0001$). The greatest absolute risk reduction was in women of 40 years or under whose risk of local recurrence at 20 years was reduced from 36% to 24% (p = 0.003) and in whom the likelihood of requiring salvage mastectomy was reduced by 41% at 10 years [4]. For patients with grade 3 disease, the addition of the boost dose reduced the risk of local relapse from 18.9% to 8.6% at 10 years ($p = 0.01$) [6]. For the study population as a whole, there was no significant difference in overall survival at 20 years (59.7% in the boost group versus 61.1% in the no boost group ($p = 0.323$)) [5]. The risk of severe fibrosis however was increased in the boost group (5.2% versus 1.8% at 20 years ($p < 0.0001$)) [5].

With regard to other indications for a tumour bed boost, recently published consensus guidelines conclude that there is no clear evidence that escalating the dose to the tumour bed reduces the risk of ipsilateral breast tumour recurrence in women whose excision margins are close but clear of ink [7]. For women with focally positive margins however, a recent analysis of outcomes in 8485 women treated with breast conservation surgery and whole-breast irradiation at the Netherlands Cancer Institute, where focal margin positivity is not an indication for re-excision and where 79% of patients receive a tumour bed boost, no increased risk of local recurrence has been found at a median follow-up of 9 years suggesting that a tumour bed boost may safely replace re-excision in this scenario [8].

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51.2 The Risks of Whole-Breast Irradiation

Alongside the local control and survival benefits of whole-breast irradiation, the EBCTCG meta-analyses have also demonstrated a small but significant increase in the risk of non-breast cancer-related mortality. The 2005 EBCTCG update demonstrated an absolute increase in non-breast cancer-related mortality of 1% at 15 years post-radiotherapy [1], the majority of which is attributable to cardiac disease [1, 9]. A recent case-control study in women treated with radiotherapy for breast cancer has demonstrated a linear relationship between the mean radiation dose to the heart and the risk of major coronary events (MCEs) (including ischaemic heart disease death, myocardial infarction and coronary revascularisation procedures) [10]. The risk of MCEs was reported to increase by 7.4% per Gray (Gy) mean heart dose with no apparent threshold below which a woman is at no risk of radiation-induced heart disease. The proportional increase in risk was similar in women with and without cardiac risk factors, and the increased risk began within the first 5 years after administration of radiotherapy. It is not yet clear which cardiac substructures, when irradiated, contribute the most to the risk of cardiovascular disease, but evidence from myocardial perfusion [11] and coronary angiography studies [12, 13] suggests that the left anterior descending coronary artery is a key structure in the pathogenesis of radiation-induced heart disease.

Death from second malignancy in the lung accounts for <10% of non-breast cancer-related deaths [1]. The relative risk of death from a second malignancy in the lung ranges from 1.5 to 2.8 at 15 years [14, 15], with odds ratios of up to 37.6 in smokers [16]. Data on secondary lung malignancy deaths in 9000 women irradiated between 1935 and 1971 [14] suggest a dose-response relationship with an incremental relative risk of 0.2 per Gy to the ipsilateral lung (equating to nine cases of second lung malignancy/year/10,000 women receiving 10 Gy to the lung and living to 10 years). The SEER registry cohort demonstrates a similar relationship between mean lung dose and risk of second lung malignancy in women irradiated between 1973 and 2001 [9].

The EBCTCG meta-analysis also demonstrated a significantly increased incidence of contralateral breast cancer in irradiated women (9.3% versus 7.5% at 15 years, $p = 0.02$) [1], with the main excess risk appearing at years 5–14 following radiotherapy. A study of 708 cases (women with asynchronous bilateral breast cancer) and 1399 controls (women with unilateral breast cancer) from the US Women's Environment, Cancer, and Radiation Epidemiology Study found that radiotherapy increased the risk of second primary contralateral breast cancer only in those irradiated under 45 years of age [17]. Although the majority of contralateral breast cancers arise in the upper outer quadrant [18], a higher proportion was found in the inner quadrants in previously irradiated women. In women aged <40 years, those who received >1 Gy of radiation to the index quadrant had a 2.5-fold greater risk of contralateral breast cancer than unexposed women (95% CI 1.4–4.5) [17]. The dose-response relationship was also significant (excess relative risk per Gy of 1.0, 95% CI 0.1–3.0). The results suggest that attempts should be made to limit the mean contralateral breast dose to <1.0 Gy in young women undergoing radiotherapy for breast cancer.

With regard to other tissues, the EBCTCG study [1] reported a 20% increase in incidence of second primary malignancies (SPM) in irradiated women compared to unirradiated women (standardised incidence ratio (SIR) = 1.20). This equates to approximately 35 second malignancies per 10,000 women at 10 years (not including lung cancers) and around 60 second malignancies including lung cancers. Significant excess risks were found for the oesophagus (SIR = 2.06), soft tissue sarcoma (SIR = 2.34) and leukaemia (SIR = 1.71), but not for melanoma, bone sarcoma, colorectal, stomach, kidney, uterus and thyroid cancers. Roychoudhuri [15] also found excess risks of myeloid leukaemia (RR = 2.0) and oesophageal cancer (RR = 2.2) in women following whole-breast radiotherapy. Two small studies have estimated sarcoma incidence following breast radiotherapy to be 0.2% at 10 years [19, 20], the majority arising in the breast and chest wall. The incidence of SPM is markedly increased in women irradiated under 40 years of age [21, 22]. With increasing use of systemic therapy in recent years, the incidence of second malignancy may increase further, with one study reporting a 4% incidence of second malignancy at 10 years following treatment with chemo- and radiotherapy [23].

Otherwise, direct irradiation of breast tissue, ribcage and muscle has been shown to increase long-term morbidity and reduce quality of life [24]. Results from the UK START trial suggest that 20% of patients experience some breast shrinkage, 40% breast hardness, 40% a moderate or marked change in breast appearance following radiotherapy and 40% some degree of chest wall discomfort [24, 25], all of which have the potential to increase long-term physical and psychological morbidity.

Given the increasing number of long-term breast cancer survivors [26], radiation-induced mortality and morbidity could impact upon millions of women worldwide. A current priority in breast radiotherapy then is to reduce long-term morbidity and mortality without compromising local control, and optimal technical approaches for doing so are discussed further below (see section 51.5.4).

51.3 Are There Groups of Women in Whom the Risks of Whole-Breast Irradiation Outweigh the Benefits?

Local recurrence rates have fallen considerably since the women in the EBCTCG meta-analysis studies were treated [27] such that the absolute gains of whole-breast irradiation are likely to be considerably lower than reported previously. The reduction in relapse risk with era of treatment is illustrated in Fig. 51.1 and is likely to reflect improvements in early diagnosis, systemic therapies and surgical and radiotherapeutic techniques [28].

To date however, it has not been possible to identify a group of women in whom the risk of local recurrence is so low as to be able to routinely omit whole-breast radiotherapy. The PRIME II study [29] randomised 1326 women of 65 years and older with ER-positive tumours <3 cm, clear margins and negative nodes treated with breast conservation surgery and endocrine therapy to whole-breast irradiation (40–50 Gy in 15–25 fractions) versus no radiotherapy. At a median follow-up of 5 years, local relapse rates were 1.3% (95% CI 0.2–2.3) in the whole-breast radiotherapy arm and 4.4% (2.4–5.7) in the no radiotherapy arm. Five-year overall survival was 93.9% (CI 91.8–96.0) which was the same in both groups. Whilst these data are encouraging that there might be a group of women in whom the local control benefits are small enough to consider omitting radiotherapy, it should be borne in mind that the life expectancy of an otherwise fit and healthy 65-year-old woman living in the UK is 16–20 years [30] such that the longer-term local control and survival benefits of breast radiotherapy remain of interest.

Other work has used molecular and/or genetic profiling to help identify women at the very lowest risk of relapse. For example, luminal A and B molecular subtypes have been shown to be associated with a lower risk of local relapse than triple-negative and Her-2-enriched molecular subtypes [31], whilst the Oncotype DX recurrence score has also been shown to be able to identify a group of women with a 10-year risk of local relapse below 5% [32]. Forthcoming research (the UK PRIMETIME study) will omit radiotherapy in women considered to be at lowest risk of relapse based on a combination of clinical features and IHC4 testing with the aim of confirming the group of women in whom radiotherapy might be safely avoided.

51.4 Dose and Fractionation

The radiation dose and fractionation used in many of the early surgery versus surgery plus radiotherapy trials for breast cancer was 50 Gy in 25 fractions [33] such that this became the standard of care for many years. More recently however, the START trials [34] and a Canadian study of hypofractionation [35] have changed the standard of care in many countries.

Four thousand four hundred fifty-one women were treated within the START trials between 1999 and 2002. All women had undergone complete surgical excision of breast cancer and were otherwise treated with chemotherapy and/or endocrine therapy according to standard protocols. Women in the START-A trial were then randomised to 50 Gy in 25 fractions over 5 weeks versus 41.6 Gy or 39 Gy given in 13 fractions over 5 weeks. Women in the START-B trial were randomised between 50 Gy in 25 fractions over 5 weeks

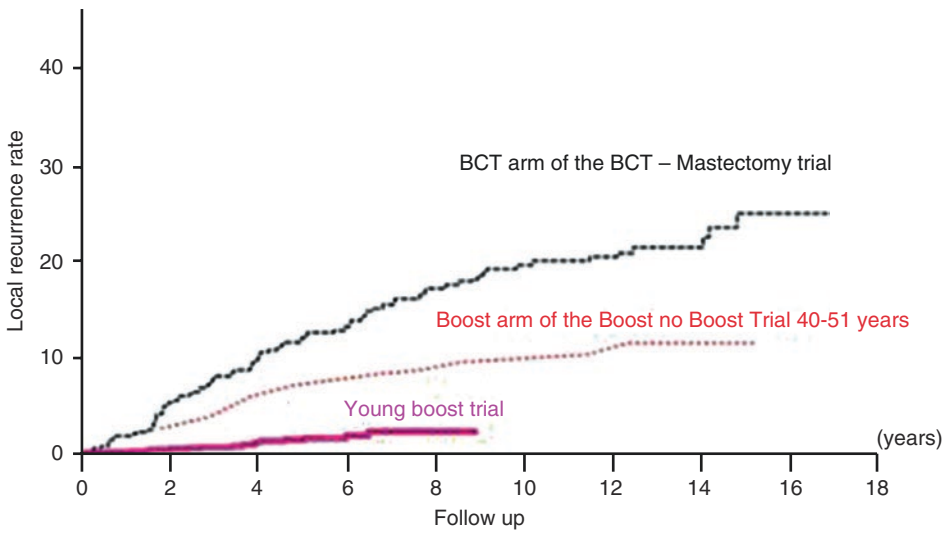

Fig. 51.1 Locoregional recurrence rates in the consecutive EORTC 10801, EORTC 22881-10882 and Young Boost Trials. Reproduced with permission (from Poortmans, The Breast, 2017, 295–307)

versus 40 Gy in 15 fractions over 3 weeks. At a median follow-up of almost 10 years, loco-regional relapse rates did not differ significantly between the groups [34]. However, breast firmness, skin changes and breast oedema (or swelling) were significantly less common in the 39 Gy group of START-A and 40 Gy group of START-B as compared to the 50 Gy group in each study.

Meanwhile, the Canadian study randomised women undergoing whole-breast irradiation to 50 Gy in 25 fractions over 5 weeks versus 42.5 Gy in 16 fractions over 3 weeks [35]. The 10-year risk of local recurrence was 6.7% in the 50 Gy in 25 fraction arm and 6.2% in the 42.5 Gy in 16 fraction arm (absolute difference 0.5%, 95% confidence interval −2.5 to 3.5). 71% of the standard fractionation patients were reported to have a good or excellent cosmetic outcome at 10 years as compared to 70% in the control group (absolute difference 1.5%, 95% confidence interval −6.9 to 9.8).

In the light of these three studies, the standard fractionation for whole-breast irradiation has in many countries become 40 Gy in 15 fractions. It is unlikely though that the limits of hypofractionation have been reached. The UK FAST study randomised 915 women with node-negative early breast cancer to 50 Gy in 25 fractions versus 28.5 Gy and 30 Gy delivered in five weekly fractions of 5.7 Gy and 6.0 Gy, respectively. At a median follow-up of 3 years, changes in photographic breast appearance were comparable between the 50 Gy in 25-fraction and 28.5 Gy in 5-fraction regimens and significantly milder than the 30 Gy in 5-fraction regimens [36]. There were only two local recurrences at 3 years, both of which were in the 50 Gy in 25-fraction arm of the study, but mature late effect and local control data are awaited.

The UK FAST-Forward study has gone on to randomise women between 40 Gy in 15 fractions and 26 or 27 Gy in 5 fractions over 1 week. This trial closed to recruitment in 2014 such that 5-year local control data will not be reported before 2019. In the meantime however, acute toxicity has been reported to be comparable between the three fractionations [37].

51.5 Technical Approaches to Whole-Breast Irradiation

51.5.1 Target Volume Definition

Most whole-breast irradiation continues to be delivered adjuvantly such that the gross tumour volume (GTV) has been excised leaving the tumour bed as a surrogate for the GTV. Studies testing the accuracy of clinical methods against orthogonal X-ray imaging localisation of clips for defining the tumour bed itself show that clinical methods result in a geographical miss and/or unnecessary normal tissue irradiation in up to 50% of patients [38–41]. To overcome the inaccuracies of clinical methods, several imaging modalities have been tested, most of which rely on the identification of seroma which may either be variable in volume [42] or, in patients who have undergone oncoplastic surgery [43], misrepresent the true tumour bed. Insertion of radio-opaque markers into the excision cavity walls overcomes some of the limitations described above. Tumour bed clips inserted according to protocol in pairs at the four radial, superficial and deep cavity margins according to a defined protocol [44] provide additional localisation information compared to kV-CT imaging alone, leading to modification of whole-breast tangential field borders in 43% of patients [45]. Clips inserted under direct vision do not migrate [45], and serial CT imaging also suggests that clips serve as stable surrogates for the TB over time [46].

The clinical target volume (CTV) for whole-breast irradiation should include the whole of the glandular and subcutaneous breast tissue although results of partial breast irradiation studies (discussed in chapter 55) suggest that, for lower-risk breast cancer, it may no longer be necessary to include the entirety of the ipsilateral breast tissue in the CTV. In any event, most protocols exclude the skin and scar from the CTV by limiting the CTV to within 5 mm of the skin surface [47].

The CTV to planning target volume (PTV) margin will depend mainly on set-up errors and respiratory motion. In the context of supine whole-breast irradiation, a number of studies [48–55] have used electronic portal imaging to quantify the extent of positional errors and patient movement. Systematic errors range from 1.0 to 14.4 mm (the worst figures being associated with a lack of arm immobilisation) and random errors from 1.7 to 5.8 mm. Intra-fraction errors range from 0.8 to 3.2 mm [48, 49, 51]. Errors vary between departments, partly due to different immobilisation devices, such that institutions are recommended to use their own set-up data if possible [56]. Data from the Royal Marsden show that changes in breast volume (peaking early in the treatment course at ~105% of the initial whole-breast volume) and set-up errors are adequately encompassed by a 5 mm margin [57, 58] (mean set-up error = 1.2 mm, standard deviation = 2.5 mm, generating a CTV to PTV margin = (2.5 × 1.2) + (0.7 × 2.5) = 4.75 mm based on the van Herk formula [59]). A further margin of 5 mm to allow for the effects of respiratory motion has been deemed adequate in the majority of patients [58] generating a total CTV to PTV margin of 10 mm.

51.5.2 Position and Immobilisation

Patient positioning and immobilisation are crucial to minimising set-up errors. A supine position on an angled board with adjustable arm supports is standard in many centres.

This set-up technique is compatible with most modern CT scanners and is associated with satisfactory set-up errors [57, 58]. The use of arm supports has been shown to be superior to using an angled board alone [54]. Coverage of lymph node regions has been shown to be more homogeneous when both arms are raised above the head as compared to a single arm being raised [60].

Patients with large pendulous breasts or large breast separations can be poor candidates for the standard supine treatment position [61] because dose distributions across the treatment volume are inhomogeneous [62] resulting in fibrosis [63] and poor cosmesis [64] and because gravity tends to pull breast tissue laterally, such that large volumes of nontarget tissue must be irradiated in order to adequately encompass target tissue [65]. Treatment of larger-breasted women in a bra has been shown to significantly reduce radiation doses to heart and ipsilateral lung albeit at the expense of increased grade 2 acute skin toxicity [66]. The reproducibility of bra-based techniques however is yet to be established.

Use of a prone treatment position in WBI can improve dose homogeneity within breast tissue [67, 68], reduce wedge requirements with consequent reduction of scattered dose and reduce dose to the lung [69–72], particularly in women with larger breast cup sizes (≥D) [70], but also in women of average breast cup size (median C) [71, 73]. However, data regarding the effects of prone positioning on cardiac doses are conflicting. Buijsen et al. demonstrated a reduction in heart V_{30Gy} from 7.3% (supine) to 2.4% (prone) in women of D cup and above [70]. However, other studies have failed to demonstrate a statistically significant reduction in mean heart dose between supine and prone free-breathing techniques with Varga et al. [74] reporting mean heart doses of 2.9 Gy for supine 3D-conformal RT and 2.2 Gy for prone treatment ($n = 83$, no significant difference) and Mulliez et al. [75] reporting mean heart doses of 2.0 Gy for supine inverse-planned multi-field IMRT and 1.5 Gy for prone tangential field treatment ($n = 60$, no significant difference). Other authors advise caution in using the prone position based on the fact that the heart falls forward in the prone position [76] such that, in smaller-breasted women (C cup and below), the prone position may even increase the heart dose as compared to supine free-breathing [77]. It should also be borne in mind that coverage of level I and II axillary lymph nodes by tangential fields is reduced in the prone position [78].

In terms of reproducibility, some centres use prone radiotherapy in routine practice and have achieved excellent reproducibility albeit using more complex verification protocols than standard electronic portal imaging [79–81] such as daily cone-beam CT [79] or imaging of breast tissue markers [80]. Other groups have reported reduced deliverability and reproducibility of prone compared to supine treatment [82–84]. In one of these studies [83], prone positioning was compared to supine voluntary breath-hold in larger-breasted women (D cup and above). The study was stopped early due to the dosimetric superiority of supine breath-hold and the poor reproducibility of prone positioning although, for most patients, the mean heart dose was below 1 Gy regardless of technique suggesting that prone treatment is a safe approach in the hands of those who can reproduce the position reliably. Insertion of radio-opaque markers into the excision cavity walls overcomes some of the limitations described above. Tumour bed clips inserted according to protocol in pairs at the four radial, superficial and deep cavity margins according to a defined protocol [44] provide additional localisation information compared to kV-CT imaging alone, leading to modification of field borders in 43% of patients [45]. Clips inserted under direct vision do not migrate [45] and serial CT imaging also suggests that clips serve as stable surrogates for the tumour bed over time [46].

51.5.3 2D vs. 3D Planning

Whole-breast radiotherapy planning techniques have improved considerably since the women included in the EBCTCG meta-analysis were treated, precipitated by demonstration of the clinical benefits of radiotherapy technological advances [85, 86] and also through implementation of technological advances into routine practice through departmental participation in clinical trials [87]. The Royal Marsden Breast dosimetry study randomised 306 women to whole-breast irradiation planned using three-dimensional intensity-modulated radiotherapy (3D IMRT) or two-dimensional radiotherapy (2D RT) using standard wedge compensators [85]. Women were treated to 50 Gy in 25 fractions with a boost to the tumour bed of 11.1 Gy in 5 fractions. At a median follow-up of 5 years, a change in photographic breast appearance was seen in 58% of the 2D RT patients compared to only 40% of the women allocated 3D IMRT, and significantly fewer patients in the 3D IMRT group developed clinically palpable induration. No significant differences were found however in patient-reported breast pain or quality of life. More recently, the Cambridge intensity-modulated radiotherapy (IMRT) study treated 1145 women between 2003 and 2007, randomising 815 women with inhomogeneous dose on standard wedged tangents to standard radiotherapy versus simple IMRT [86]. Fewer patients in the simple IMRT group (57%) developed suboptimal overall cosmesis compared to those treated with standard RT (63%), but there were no differences in breast shrinkage, firmness or swelling between the groups. Once again, patient-reported outcome measures (PROMs) (assessed using global health and breast symptom-specific questionnaires) showed no benefit of simple IMRT over standard radiotherapy [88]. Across the whole study population, the overall rate of adverse

outcomes reported using PROMs was low at 5 years (6% reported breast pain, 4% skin problems, <0.5% breast swelling, 15% change in breast appearance, 13% breast shrinkage and 8% breast firmness).

51.5.4 Heart-Sparing Whole-Breast Radiotherapy Techniques

As discussed in Sect. 51.2, the main mortality risk from whole-breast irradiation is radiation-induced heart disease. Although, in the majority of women, the benefits of whole-breast irradiation will outweigh the risks [89], data relating the likelihood of major coronary events to mean heart dose [10] suggests that we should maintain heart doses as low as reasonably achievable. Simple heart-sparing approaches include optimising beam angles and/or using multileaf collimation shielding to reduce the volume of heart tissue included in tangential fields. Where such approaches are used, it is recommended that target volumes be formally delineated to ensure that the tumour bed and surrounding tissues receive an adequate radiation dose [90].

IMRT and arc therapy approaches can be used to conform high-dose regions more closely to the breast CTV so as to reduce the volume of heart tissue treated to higher doses. Jin et al. [91] reported values for the volume of heart receiving 20 Gy or more (V_{20Gy}) of 5.6% for standard tangential wedged fields, 4.3% for forward-planned IMRT (F-IMRT), 2.1% for seven-field IMRT (7-IMRT) and 2.0% for tangential inverse-planned IMRT (T-IMRT) suggesting a benefit from the inverse-planned approaches. However, the more complex the field arrangement, the larger the low-dose bath of radiation such that the mean heart dose may conversely be increased by multi-field IMRT approaches. Predominantly tangential approaches may be more successful in reducing mean heart dose with the same authors reporting mean heart doses of 3.7 Gy for tangential fields, 3.2 Gy for F-IMRT and 4.4 Gy for seven-field IMRT, whilst T-IMRT reduced mean heart dose to 2.2 Gy.

The advantages and disadvantages of prone positioning on heart doses have been discussed in Sect. 51.5.2. A simpler and perhaps more widely implementable approach is to treat patients in deep-inspiratory breath-hold (DIBH) whereby the diaphragm pulls heart tissue inferiorly, posteriorly and medially away from the whole-breast radiotherapy fields. DIBH techniques reduce mean heart dose by around 50% [92–95]. For example, Wang et al. [94] reported a reduction in mean heart dose from 3.2 Gy using forward-planned IMRT in free-breathing to 1.3 Gy for forward-planned IMRT in breath-hold.

There are several available technical approaches to delivering radiotherapy in breath-hold ranging from maintaining the patient in breath-hold externally (e.g. using the Active Breathing Coordinator™ (ABC) device (Elekta, Crawley, UK)) to delivering radiotherapy only when the patient is in the inspiratory phase of their breathing cycle (e.g. the Varian Real-Time Position Monitoring gating solution (Varian Medical Systems, Palo Alto, USA)). Gating solutions can be delivered with or without the use of goggles providing visual feedback to the patient on where they are in their breathing cycle. Alternative technologies include AlignRT® (Vision RT Ltd., London, UK) which uses 3D optical surface imaging to verify that the patient's breath-hold is consistent. There are capital and resource costs associated with each of these techniques however such that, in some health economies, the implementation of breath-hold techniques has been slow (Royal College of Radiologists' Breast Radiotherapy Audit, UK, 2012, Imogen Locke, personal communication). In the light of this, a simple voluntary breath-hold technique has been developed which requires little more than a standard linear accelerator and a felt-tip pen [96]. This voluntary breath-hold technique has been shown to be as heart-sparing and reproducible as an ABC-based technique, as well as being faster to deliver and more acceptable to patients and radiographers [97].

Proton beam therapy (PBT) is not yet widely available for the treatment of breast cancer outside Northern America. Planning studies have shown that PBT can reduce mean heart doses in women undergoing radiotherapy to the whole breast (mean heart dose 12 Gy for 3D-conformal RT versus 1 Gy for PBT) [98]. With regard to other toxicities, the only phase I randomised comparison of PBT versus photon treatment is in women undergoing accelerated partial breast irradiation to 32 Gray in eight fractions delivered twice daily). This reports, at a median follow-up of 7 years, increased skin telangiectasia (69% versus 16%, $p = 0.0013$) and pigmentation changes (54% versus 22%) for PBT versus photon therapy [99]. As yet there is no equivalent dataset for women undergoing standardly fractionated whole-breast irradiation, but current trials are ongoing. In terms of cost-effectiveness, the earliest report comparing PBT and conventional whole-breast irradiation suggested that PBT may be cost-effective in women at highest risk of cardiac toxicity [100]. More recent work however suggests that the PBT is only modestly more cost-effective than standard photon-based whole-breast irradiation [101].

Alternatives to whole-breast irradiation such as partial breast irradiation [102] (including intraoperative radiotherapy) are also able to reduce mean heart doses and are discussed in Chaps. 55 and 56, respectively.

51.5.5 Verification

The UK Intensity-Modulated Partial Organ Radiotherapy (IMPORT) trialists have compared breast radiotherapy verification techniques, firstly establishing the feasibility of

using implanted markers to verify the position of the tumour bed during a course of breast radiotherapy [103]. Gold seeds were useable for verification in 42/32 (98%) patients and, using either an extended no-action level (eNAL) protocol [104] or an online correction protocol, were compatible with a set-up margin of 5 mm (i.e. a total CTV to PTV margin of 10 mm including respiratory motion). More recent work from the same group suggests that eNAL protocols including a total of five imaging sessions (within a 15 fraction course of breast radiotherapy) can reduce total CTV-PTV margins to 6 mm compared to a no-correction or no-action level protocol [105]. Implanted markers can be imaged using a number of technologies including megavoltage CT and kilovoltage cone-beam CT. Titanium clips are most visible on the latter and can be satisfactorily imaged for the purposes of set-up verification using partial arc scan geometries which are more compatible with supine set-up techniques than full arc scans [106]. With regard to the clinical benefits of pursuing a more rigorous verification protocol, reduced CTV to PTV margins have been shown to be associated with modest reductions in doses to breast, lung and heart tissues [107].

Conclusions

For the majority of women, the benefits of whole-breast irradiation continue to outweigh the risks although work continues to identify those women whose risk of relapse is low enough that the irradiated volume can be reduced or in whom radiotherapy can be avoided altogether. Where whole-breast irradiation is given, the majority of centres continue to treat supine, using implanted markers to aid target volume delineation and using techniques such as breath-hold to reduce heart doses in left breast-affected women. Hypofractionation together with three-dimensional planning techniques and improved verification have all helped to reduce the acute and late side effects of whole-breast irradiation.

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Whole-Breast Radiation Following Breast-Conserving Surgery in Noninvasive Cancer

52

Beryl McCormick

52.1 Lobular Carcinoma In Situ

Lobular carcinoma in situ (LCIS) is considered a risk factor for the development of a future cancer in either breast, once the LCIS has been identified on breast biopsy. LCIS identifies a patient as having an increased risk, and may be the gateway into special surveillance programs; however, there is no data supporting treatment of the LCIS diagnosis with breast radiation.

In 1992, a subtype of LCIS, labeled pleomorphic LCIS, was first described by Eusebi et al. [1]. Since that time, a number of articles have been published regarding this entity; although its pathology, imaging, and molecular features are well described, there is no data on outcomes with specific treatments. Please refer to the pathology section of this textbook for more information.

52.2 Ductal Carcinoma In Situ

Ductal carcinoma in situ (DCIS) is a common breast cancer diagnosis in regions of the world with breast cancer screening programs or access to mammography for screening. In the United States in 2013, this diagnosis accounted for 20–25% of all new breast cancers [2]. With the recognition that results of the two local treatments were similar in early prospective randomized trials comparing breast-conservation surgery and whole-breast radiation to mastectomy for invasive cancers [3, 4], the practice of offering women with DCIS a similar local treatment option developed.

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52.2.1 Early Prospective Clinical Trials

The National Surgical Adjuvant Breast and Bowel Project (NSABP) cooperative group was the first to initiate a prospective clinical trial assessing the value of whole-breast radiation after breast-conservation surgery for women with DCIS. Part of the rationale for this new study followed a central pathology review of women in the practice-changing B-06 trial, comparing mastectomy, breast-conservation surgery, and breast-conservation surgery with whole-breast radiation for women with early invasive breast cancers. Seventy-eight women enrolled in that study were actually found to have a diagnosis of DCIS on central pathology review, and in a separate report the NSABP noted "local breast recurrences were similar for women with DCIS and those from this cohort" (with invasive disease), strongly suggestive of a similar approach with breast-conservation surgery for both diseases [5].

The trial design for the NSABP B-17 study compared whole-breast radiation to none, following breast-conservation surgery, for 818 women with DCIS; reflecting the era of the trial, women were eligible whether the method of detection of their DCIS was by physical exam or by mammogram only. All pathology subtypes of DCIS were included, with the only pathology stratification factor being the presence or absence of LCIS along with the DCIS. Tumor-free margins were also required, and the cohort randomized to receive whole-breast radiation was given a dose of 50 Gy without a boost. Figure 52.1 shows the results of this trial with a median follow-up time of 90 months [6]. The women assigned to receive radiation had a highly significant decrease in ipsilateral breast tumor recurrence, both invasive and in situ, although no differences in survival endpoints were observed.

Shortly after, the European Organisation for Research and Treatment of Cancer (EORTC) reported the results of a nearly identical study, EORTC 10853, of 1010 women with DCIS, again comparing radiation to none following

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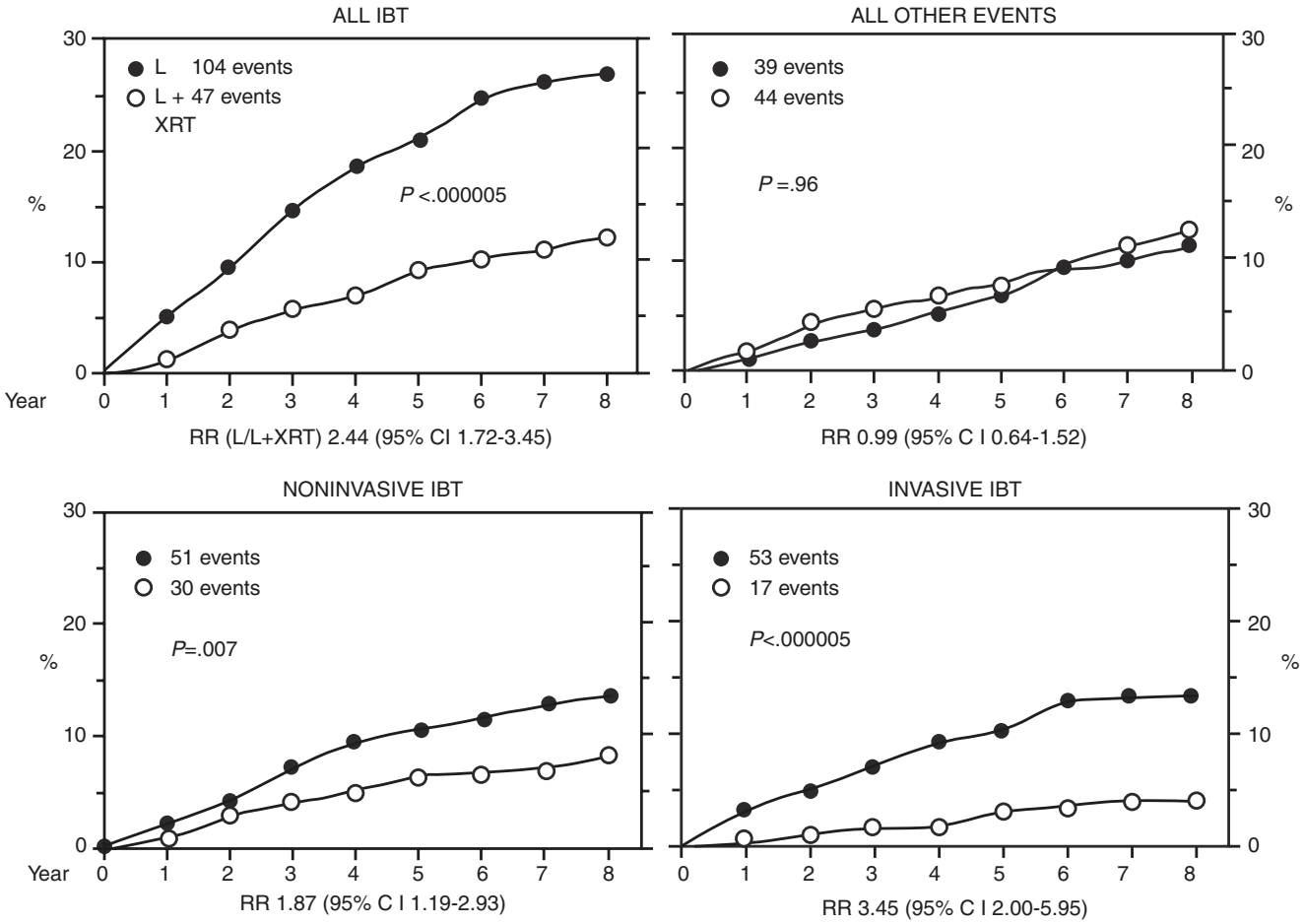

Fig. 52.1 Cumulative incidence of all ipsilateral breast tumors, by pathology type, and all other breast events in NSABP B17. *Open circles* represent patients who were randomized to receive whole-breast radiation, and *closed circles* those treated with surgery only

breast-conservation surgery. Consistent with the results of the B-17 trial, a 47% reduction in local failure in the ipsilateral breast was noted at 10 years for those women assigned to the whole-breast radiation arm of the trial. The dose was 50 Gy without a boost. Figure 52.2 from that study shows the positive effect of the radiotherapy in all clinical and pathologic subsets of patients included in the study [7].

52.2.2 Clinical Trials Focused on Subtypes of DCIS

The Swedish Breast Cancer Group opened a study of whole-breast radiotherapy versus none, focused on those women in the country's national screening program; as a result of the trial design, almost 80% of the women enrolled had no symptoms, but only screen-detected DCIS. There were 1067 women enrolled in the trial, and those assigned to receive whole-breast radiotherapy were given a dose of 50 Gy in 25 treatments, or 54 Gy in "two series with a gap of two weeks." Fig. 52.3 demonstrates the effect of radiotherapy on reducing the risk of local recurrences, with a median follow-up of 5.2 years for the women on study [8]. This decreased risk is consistent with both the EORTC and the NSABP B 17 studies.

In 1997 the Eastern Cooperative Oncology Group (ECOG) opened a clinical trial with prospective observation only, confined to specific risk stratum of DCIS. The low-intermediate group consisted of women with biopsy-proven low- or intermediate-grade DCIS, which was nonpalpable, did not exceed 2.5 cm in greatest diameter, and had a minimal margin width of 3 mm. ECOG acknowledged the results of prior prospective randomized trials favoring the use of radiotherapy but hypothesized that "a combination of size of lesion, grade, and surgical margin width might define a subset of patients at low risk of local failure without radiation" [9]. The study required

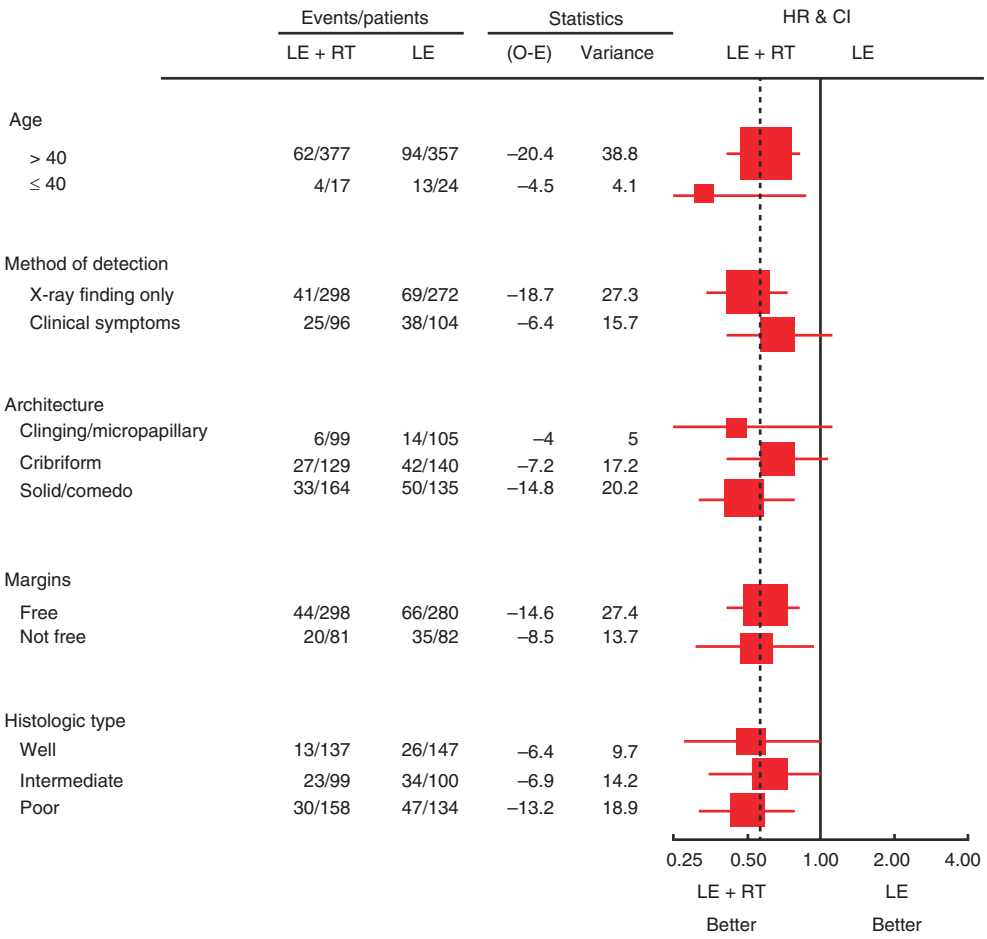

Fig. 52.2 Effect of radiotherapy on local control by subgroup in European Organisation for Research and Treatment of Cancer (EORTC) 10,853. *LE + RT* patients randomized to receive radiotherapy, *LE* those with surgery only

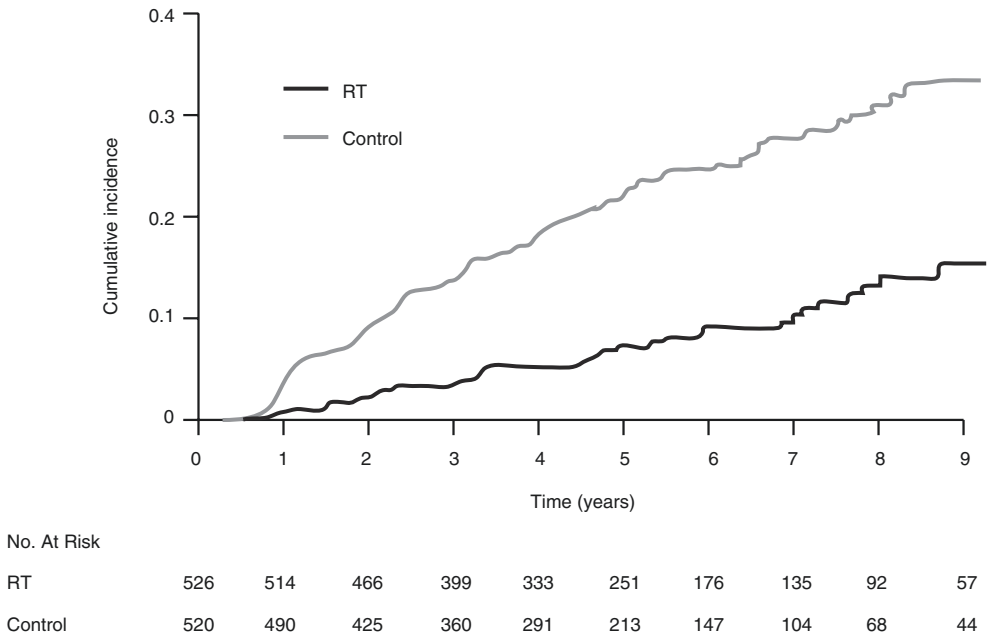

Fig. 52.3 Cumulative incidence of all ipsilateral recurrences in the SweDCIS trial. $P = <0.0001$ log-rank test

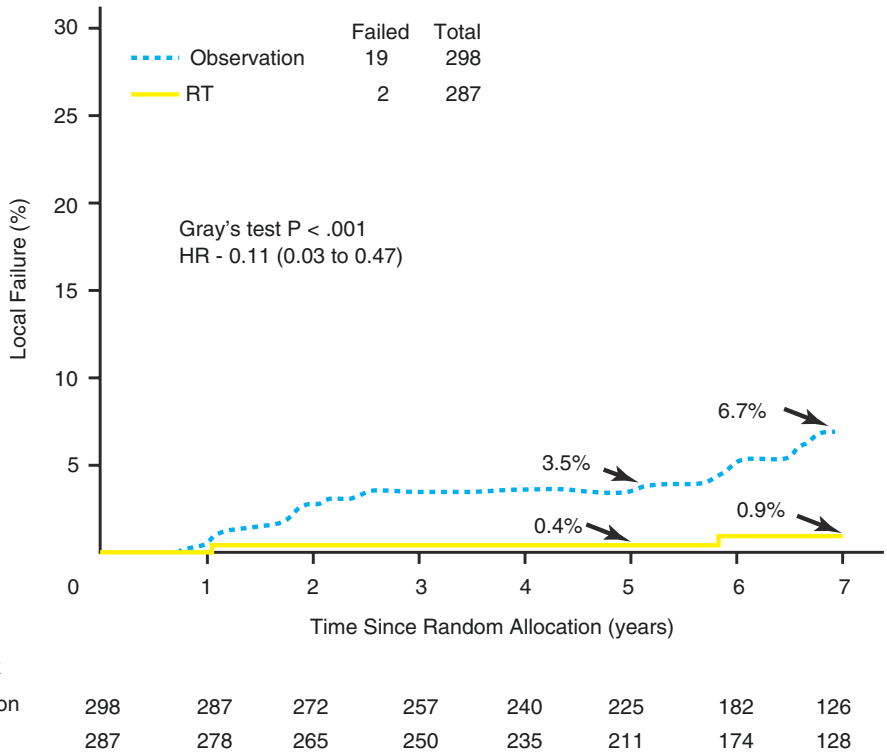

Fig. 52.4 Local failure in the ipsilateral breast of patients in the Radiation Therapy Oncology Group (RTOG) 9804 trial

central pathology review and allowed for the use of tamoxifen, which was used by 30% of patients on the study. A second, high-risk stratum consisted of women with high-grade DCIS, also nonpalpable with a maximum size of 1 cm and the same margin criteria as the "low-intermediate"-risk group. With a median follow-up time of 12.3 years, the local recurrence at 12 years was 14.4% in the low-intermediate-risk stratum and 24.6% in the high-risk stratum. The authors concluded that "individual patients and their physicians will need to decide if these 12-years risks are acceptable and to judge whether or not to add adjuvant treatment after surgical incision" [10].

The Radiation Therapy Oncology Group (RTOG) focused on the same low-intermediate-risk subset of DCIS patients as the ECOG study above, but this group's trial design was a prospective randomized study comparing radiation to none, for nonpalpable DCIS, also excised with a margin of 3 mm or greater, and measuring no larger than 2.5 cm in greatest diameter. Based on results of the NSABP's B-24 DCIS study showing an advantage in terms of improved local control with the addition of tamoxifen to whole-breast radiation [11], the study opened requiring tamoxifen use for 5 years in both study arms. The use of tamoxifen was made optional with the publication of the UK, Australian, and New Zealand radiotherapy and tamoxifen trial [12], resulting in an overall use of tamoxifen in the RTOG study of about 70% [13].

For those women assigned to receive radiotherapy in the RTOG study, the dose was originally 50 Gy in 25–28 fractions, but an amendment allowing for the use of 42 Gy in 16 fractions was added. Although this study was closed prior to meeting its targeted accrual, the addition of whole-breast radiation in reducing the rate of ipsilateral local failure was highly significant. Figure 52.4 shows those results, with a median follow-up time of 7.17 years. Disease-free and overall survival in the RTOG study and in all the prospective trials discussed above showed no differences with or without the use of whole-breast radiation.

52.2.3 Decision Making for Whole-Breast Radiation Following Conservation Surgery

As more information about the various subtypes of DCIS is appreciated, particularly with regard to outcomes from interventions, it becomes clear that the conclusion of the early prospective clinical trials, that radiotherapy improves local control and should be the standard of care, is open to some interpretation; a thoughtful discussion between the patient with DCIS and her doctors to explain risks and benefits of whole-breast RT is required. Patients need to understand that radiotherapy always lowers the risk of ipsilateral local failure, but has no measurable impact on breast cancer-related survival.

Clearly the risk of local failure is influenced by patient age, size and presentation method of the DCIS, extent of surgical resection, and pathologic characteristics of the DCIS subtype. What is the specific risk for a given patient? Several nomograms have been developed using clinical and pathologic parameters to estimate local recurrence risk; the Memorial Sloan Kettering nomogram, using ten parameters, has been validated in several DCIS populations and is a good example of such a tool [14]. Presenting the patient with a good estimate of her risk for recurrence is important when discussing the risks versus the benefits of radiation. Different patients view risk differently; two patients with the same nomogram score will often choose different local treatments. For those that choose to forego whole-breast RT, careful follow-up must be emphasized.

52.2.4 Radiation Techniques and Common Side Effects

Whole-breast radiotherapy for women with DCIS is similar to that for a patient with an early invasive cancer. The entire breast receives the dose planned through two tangent fields; 50 Gy in 25 fractions over 5 weeks' time has been the standard for many years. But the RTOG trial discussed above allowed for hypofractionation of 42 Gy in 16 fractions, and neither of the two local failures observed in the RTOG study were in the women treated with this fractionation [13]. Of note, none of the prospective randomized trials discussed above used a boost, or additional radiation to the lumpectomy bed following the completion of the whole-breast radiotherapy portion, and thus this is not needed if negative margins have been achieved.

Careful treatment planning is essential, since the heart and lungs are normal organs located close to the target breast volume. Planning should begin with a noncontrast computed tomography simulation, to localize breast target volume, and the location of the lungs, heart, and other normal organs with the patient in the treatment position. This can be either supine or prone or in the decubitus position, depending on her anatomy and the experience of the department. Great care is taken to reproduce the patient's position each day for treatment, so the treatment beams are targeting the breast with minimal dose to the ipsilateral lung. In most cases, the heart can be shielded from the primary beams, either with favorable anatomy or with special heart-avoidance techniques such as deep inspiration breath holds [15].

The most common systemic side effect is fatigue. Other common side effects are limited to the breast being treated and include temporary skin discoloration and dryness, similar to a mild sun exposure; hyperemia; mild swelling or edema of the breast; and sometimes myositis of the chest wall muscles behind the breast. Lung and heart side effects and late effects are rare with good treatment planning. Although not usually evident unless further surgery is required, radiation also can affect the elasticity of the skin, and impact on the need for breast reconstruction if a mastectomy is required later to address a local failure in the treated breast.

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Postmastectomy Radiation Therapy of Early Breast Cancer

53

Birgitte Vrou Offersen and Mette Skovhus Thomsen

53.1 Introduction

Postmastectomy radiation therapy (PMRT) in high-risk breast cancer patients reduces the risk of locoregional recurrence and improves disease-free and overall survival. Three hypotheses have been proposed regarding the natural history of early breast cancer. The first hypothesis was proposed by Halsted in 1894 and stated that the tumour spreads from the primary site either by direct permeation or through the lymph nodes to distant sites, thus emphasizing the importance of optimal local therapy [1, 2]. The systemic hypothesis was proposed by Fisher in 1980 and stated that the disease has already given rise to distant spread at the time of clinical presentation [3] and that the lymph nodes were a marker of but not a source of distant spread. Thus systemic therapy was the treatment of choice. In 1984 data from Koscielny et al. challenged the systemic hypothesis, because the risk of distant metastases strongly correlated with tumour size in patients treated with locoregional therapy but not chemotherapy [4]. Therefore the spectrum hypothesis was proposed in 1994 by Hellman [5], and it states that "metastases are a function of tumour growth and progression. Lymph node involvement is of prognostic importance not only because it indicates a more malignant biology, but also because persistent disease in the lymph nodes can be the source of distant disease" [5]. This implied that optimal locoregional control is of importance also for the risk of distant spread.

53.2 Value of PMRT in High-Risk Breast Cancer Patients

The value of PMRT has been evaluated in several randomized trials conducted over the last 60 years, but it was only in 2005 established in an overview analysis that PMRT in addition to improved locoregional control also results in improved overall survival [6]. Three well-conducted randomized trials dominated the results in the overview analysis, two from the Danish Breast Cancer Cooperative Group (DBCG), the DBCG 82 b&c trials, and one from Vancouver, Canada [7–9]. These three trials were initiated because adjuvant chemotherapy became standard therapy for high-risk premenopausal patients in the late 1970s and tamoxifen became standard in high-risk postmenopausal patients, and since no overall survival gain had been documented from PMRT, it was expected that PMRT was no longer necessary.

The DBCG 82 b trial was a Danish multicentre randomized trial including 1708 high-risk premenopausal patients treated with CMF and randomized to +/−PMRT in the period 1982–1989 [7]. All patients had a partial axillary dissection with median 7 nodes removed. The high-risk criteria were tumour size larger than 5 cm, and/or positive axillary nodes, and/or invasion of the skin or pectoral fascia. The CMF was given intravenously every 4 weeks for eight cycles in patients randomized to PMRT and nine cycles in patients randomized to no PMRT. The target of the PMRT was the chest wall and regional lymph nodes in the axillary and supra-/infraclavicular areas and ipsilateral internal mammary nodes. The dose prescribed was 48–50 Gy in 22–25 fractions in 5–51/2 weeks. With 10-year median follow-up, the locoregional recurrence rate (first site of recurrence alone or together with distant failure) was 9% in PMRT + chemotherapy patients in contrast to 32% in patients treated with chemotherapy alone. The corresponding survival rates were 54% and 45%, respectively, in favour of PMRT + chemotherapy ($P < 0.001$).

In parallel to the DBCG 82 b trial, the DBCG 82 c trial was conducted in 1982–1989 [8]. This study included 1375 high-risk postmenopausal patients with age <70 years but otherwise the same risk criteria as for the DBCG 82 b trial. The surgical strategy was the same as in the DBCG 82 b trial, but the systemic therapy was tamoxifen 30 mg for 1 year. With median follow-up of 10 years, the locoregional recurrence rate was 8% in PMRT + tamoxifen patients compared to 35% in tamoxifen-only patients. The corresponding

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10-year survival rates were 45% and 36%, respectively, in favour of PMRT + tamoxifen ($P = 0.03$).

Both DBCG trials have been criticized for the limited extent of surgery with only median 7 removed nodes and also for the inferior systemic therapy since CMF is considered low-dose-intensity chemotherapy and tamoxifen for 1 year is not sufficient. It was therefore an open question if the benefit of the PMRT was due to compensation for suboptimal surgery and/or suboptimal systemic therapy or if the PMRT was an independent contributor to locoregional tumour eradication.

The recurrence pattern in the DBCG 82 b&c trials has been reported with 18-year median follow-up, and compared to patients not receiving PMRT, patients receiving PMRT had significantly fewer locoregional recurrences, lower risk of distant metastasis after locoregional recurrence, lower risk of simultaneous locoregional recurrence and distant metastasis and lower risk of any distant metastasis [10]. Therefore these data supports the spectrum hypothesis proposed by Hellman, by demonstrating that optimal locoregional control results in fewer distant failures.

Similar to the DBCG 82 b trial, a trial was conducted in Vancouver, where 318 premenopausal high-risk patients were treated with CMF and randomized to +/−PMRT in the period 1978–1986 [9]. Only patients with stages I and II with positive nodes were included. A median number of 11 nodes were removed from levels I and II. CMF-based therapy was given for 12 months initially, but from 1981 it was modified to 6 months. The PMRT was delivered between the fourth and fifth cycle of chemotherapy, and the chest wall received 37.5 Gy and the mid-axilla and internal mammary nodes 35 Gy, all in 16 fractions using a cobalt 60 unit. After 15-year median follow-up, the locoregional recurrence rate was 13% for patients treated with PMRT + CMF versus 33% in CMF-only patients ($P = 0.003$). The corresponding survival rates were 54% versus 46%, respectively ($P = 0.07$).

Other randomized trials testing PMRT in patients also treated with systemic therapy have been reported [11–18]; however, it is difficult to compare the trials directly because differences are seen in radiation doses, number of fractions, treatment techniques, use of megavoltage or orthovoltage equipment, timing of radiation and systemic therapy as well as variation in the systemic therapy (different drugs, dose intensity, duration). But overall the trials demonstrated a reduction in locoregional recurrence risk with PMRT, whilst the gain in overall survival was not always clear. This may however be attributable to relatively few patients in the trials and differences in patient selection criteria and observation time and perhaps also because some studies may have used treatment techniques which resulted in increased risk of heart disease and heart death [19]. Importantly the three trials demonstrating the largest survival gain from PMRT (the DBCG 82 b&c trials and the Vancouver trial) did not find any excess cardiac mortality [9, 20, 21].

53.3 Overview Analyses of Effect of PMRT

In 2005, an overview analysis based on individual patient data from randomized PMRT trials was published [6], and in 8500 patients operated with mastectomy and axillary clearance for lymph node-positive breast cancer and randomized to +/− PMRT, a significant reduction in local recurrence at 5, 10 and 15 years was identified. Local recurrence at 5 years was seen in 22.8% of patients with no PMRT in contrast to 5.8% with PMRT; thus the absolute gain was 17.1%. The 15-year absolute gain in breast cancer mortality was 5.4% (risk reduction from 60.1% with no PMRT versus 54.7% with PMRT). Despite these results many guidelines have been reluctant to recommend PMRT to patients operated for pN1 disease (one to three positive nodes) [22–25]. Therefore a meta-analysis focusing on the gain from PMRT in pN1 patients was published in 2014 [26]. The analysis was based on individual data from more than 3700 patients operated with axillary dissection of at least levels I and II and irradiated with PMRT including the chest wall and the supraclavicular and/or axillary fossa and the internal mammary nodes. For 1314 patients with pN1 disease, PMRT reduced the locoregional recurrence ($P < 0.0001$), the overall recurrence (relative risk (RR) 0.68, 95% CI 0.57–0.82, $P < 0.0001$) and the breast cancer mortality (RR 0.80, 95% CI 0.67–0.95, $P = 0.01$). Of these, 1133 patients were treated in trials with systemic therapy (CMF or tamoxifen), and in these patients PMRT reduced locoregional recurrence ($P < 0.0001$), overall recurrence (RR 0.67, 95% CI 0.55–0.82, $P < 0.0001$) and breast cancer mortality (RR 0.78, 95% CI 0.64–0.94, $P = 0.01$). For 1772 patients with more than three macrometastases, PMRT reduced locoregional recurrence ($P < 0.0001$), overall recurrence (RR 0.79, 95% CI 0.69–0.90, $P = 0.0003$) and breast cancer mortality (RR 0.87, 95% CI 0.77–0.99, $P = 0.04$) [26]. The relative gain from PMRT using any first recurrence and breast cancer mortality as endpoints was the same in patients with one versus two to three macrometastases, and it was not possible to identify a subgroup of patients in the pN1 cohort who did not have a significant gain from PMRT.

It should be noted that the patients included in the trials of this meta-analysis were treated decades ago according to the routines of those days, and therefore they may not reflect the patients of today. For example, patients today most often present with screen-detected clinically node-negative cancer, and therefore they are operated with sentinel node biopsy, which was not used previously. Also modern systemic therapy is more effective than CMF or 1-year tamoxifen, and therefore the recurrence risk of modern patients may be smaller than in the meta-analysis, resulting in a smaller absolute gain in recurrence from PMRT. On the other hand, due to higher quality in PMRT today with detailed target definition and CT-based dose planning, the proportional gain from PMRT may be higher than demonstrated in the meta-analysis.

However, as data is maturing and accumulating in favour of PMRT in all node-positive patients and patients with large tumours pT3pN0 (>50 mm), European guidelines now recommend PMRT to these patient categories [27, 28].

53.4 What Is the Target of PMRT?

Since the turn of the millennium, most RT departments have changed to CT-based dose planning. Previously simulator-based techniques were used based on bony structures to guide the field arrangement. Modern PMRT is based on target definition on CT scans of the patient in treatment position. A consensus guideline for target delineation in early breast cancer was reached in ESTRO (the European Society for Radiotherapy and Oncology) in 2015, and it has gained use in most European countries [29]. According to this guideline, lymph node volumes are delineated around the large veins, because lymph nodes follow the veins. There is general consensus that in PMRT the target includes the chest wall and the regional nodal areas around the breast region, thus levels I, II, III and IV (previously called the supraclavicular lymph nodes), the interpectoral nodes and the internal mammary lymph nodes. Depending on the extent of the lymph node dissection, inclusion of levels I and II varies, for example, in some institutions levels I and II are omitted from the RT fields if >10 lymph nodes were removed from a node-positive axilla.

The inclusion of the internal mammary lymph nodes (IMN) and the medial supraclavicular lymph nodes has been in focus recently. Three large studies have investigated the benefit on recurrence in patients with and without RT of these lymph nodes [30–32]. The EORTC 22922/10925 trial randomized 4004 patients (25% had PMRT) to +/− RT of the internal mammary nodes and the medial supraclavicular lymph nodes in the period 1996–2004 [30]. At 10 years, improvements of 1.6% in overall survival (hazard ratio (HR) 0.87, 95% CI 0.76–1.00, $P = 0.05$), and 1.9% in breast cancer mortality (HR 0.82, 95% CI 0.70–0.97, $P = 0.02$) were observed with RT. Overall the study population had a low risk of IMN metastasis, because 44% had node-negative disease but central/medial tumour location and 43% had pN1 disease. In the MA.20 trial, only breast-conserving therapy was provided (thus no PMRT), and 1832 patients were randomized to +/−regional nodes RT including the IMN [31]. At 10 years, locoregional disease-free survival was improved by 3.0% (HR 0.59, 95% CI 0.39–0.88, $P = 0.009$), and distant disease-free survival was improved by 3.9% (HR 0.76, 95% CI 0.60–0.97, $P = 0.03$). However, no significant improvement was seen in overall survival (1.0%, HR 0.91, 95% CI 0.72–1.13, $P = 0.38$) or breast cancer mortality (2.0%, HR 0.80, 95% CI 0.61–1.05, $P = 0.11$). The DBCG-IMN study was not randomized but a nationwide prospective population-based cohort study in 3089 patients, and 66% had PMRT [32]. In 2003, the DBCG RT committee decided that all patients operated for a right-sided lymph node-positive or T3N0 breast cancer should receive RT of the IMN in addition to their PMRT, whilst patients treated for a left-sided breast cancer should not receive RT to the IMN. This was decided because at that time no data showed a gain in prognosis from RT of the IMN, and in left-sided patients, RT of the IMN would cause relatively high dose in the heart, thus increasing the risk of heart disease. The analysis included patients treated in 2003–2006 in Denmark. After a median of 8.9 years, the 8-year overall survival rates were 75.9% with IMN RT versus 72.2% without IMN RT. The adjusted HR for death was 0.82 (95% CI 0.72–0.94, $P = 0.005$). Breast cancer mortality was 20.9% with IMN RT versus 23.4% without IMN RT (adjusted HR 0.85, 95% CI 0.73–0.98, $P = 0.03$). The risk of distant failure was 27.4% with IMN RT versus 29.7% without IMN RT (adjusted HR 0.89, 95% CI 0.78–1.01, $P = 0.07$). The effect of IMN RT was more pronounced in patients with high risk of IMN metastasis, and no subgroups could be identified, where the IMN RT could be omitted. A meta-analysis on these studies is awaited, but many RT centres have changed their guidelines to recommend IMN RT based on these data.

53.5 Dose and Fractionation

Traditionally the dose for PMRT has been 45–50 Gy in 25–28 fractions of 1.8–2.0 Gy per fraction, 5 fractions per week. If the surgery was no radical, an additional boost of 10–16 Gy in 5–8 fractions was added. However, after the publication of results from patients operated with breast-conserving technique mostly for node-negative disease from Canada and England, where shorter fractionation schemes were used (40–42.5 Gy in 15–16 fractions, 2.67 Gy per fraction), some countries (England and Holland) have started a more widespread use of the shorter fractionation for PMRT also [33, 34]. This is based on extrapolation of results from patients treated with whole breast radiotherapy and only to a very limited extent patients operated with mastectomy. No randomized data supports the use of hypofractionation (use of doses >2.0 Gy per fraction) in PMRT patients, so trials testing it are awaited.

53.6 Treatment Technique

The treatment techniques for PMRT have changed significantly during the previous decades reflecting the technological development from orthovoltage equipment and cobalt units to megavoltage electron accelerators with multi-leaf collimators (MLC) and advanced imaging devices. In the early days of PMRT when the treatment fields were planned

on a simulator, the techniques for accelerator treatment were typically a combination of electron and photon fields [35–37]. Treatment techniques with electron fields only, either multiple static fields [38] or an arc technique [39], have also been applied. In 2002 Pierce et al. presented a dosimetric comparison of seven different techniques for PMRT of the chest wall and IMN [40]. Their conclusion was that no single treatment technique fulfilled the criteria of good target coverage with minimal organ at risk exposure. However, of the techniques studied, they found that the best compromise was obtained with the partially wide tangent technique (PWTF). This was also found in a later comparison between the PWTF technique and the technique combining electron and photon fields [41]. Furthermore, with CT-based planning the former technique was faster both to plan and when the fields were delivered at the accelerator. Figure 53.1 shows an example of the widely used mono-isocentric technique with two wide tangential half-beam fields covering the chest wall and IMN

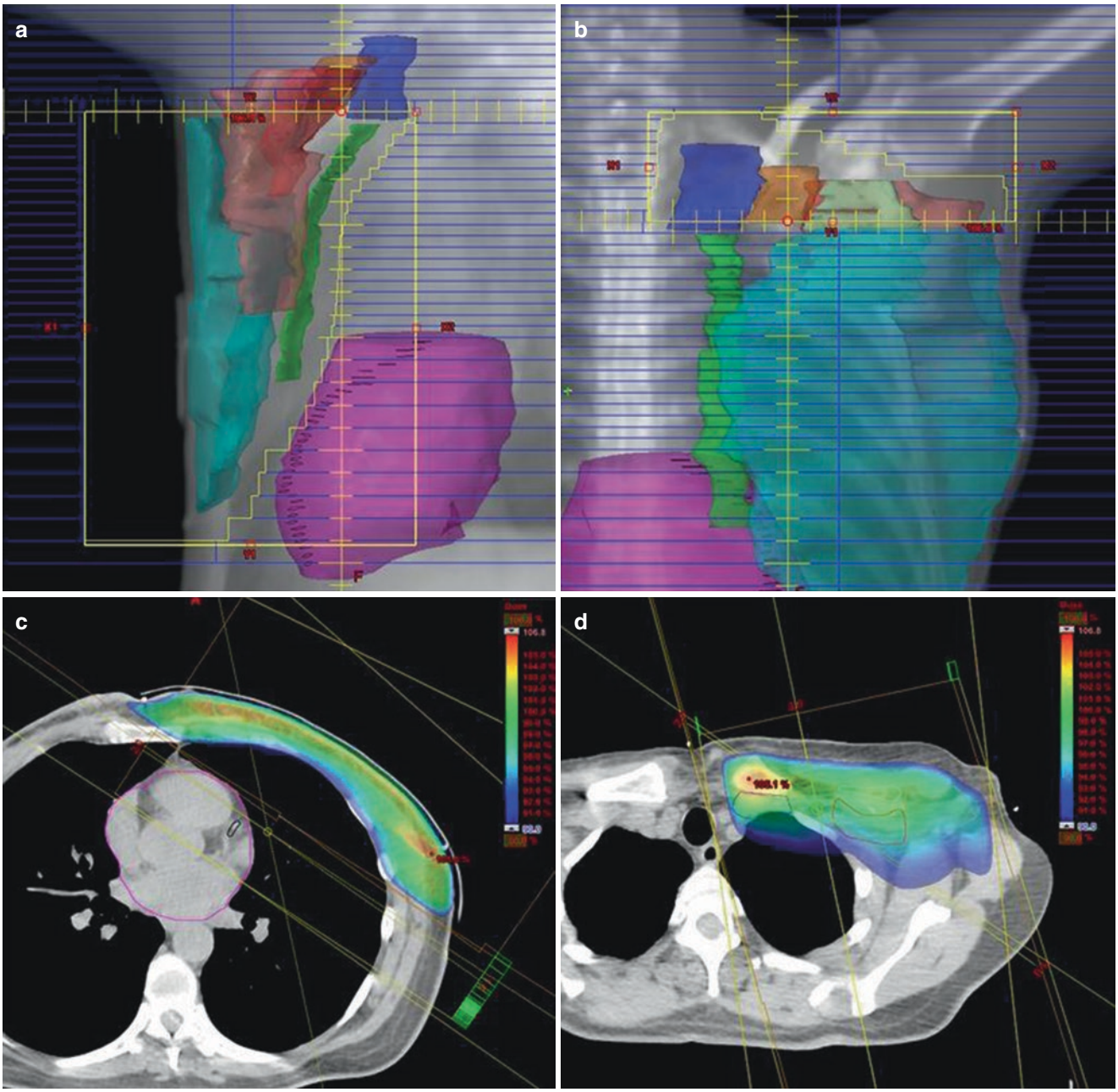

Fig. 53.1 The mono-isocentric PWTF technique with beam's eye view of one of the half-beam tangential fields (**a**) and the slightly oblique AP half-beam field (**b**). The transversal CT slices show the dose distribution at the heart level (**c**) and in the supraclavicular region (**d**). The structures shown are CTVn_L1, *pink*; CTVn_L2, *red*; CTVn_L3, *orange*; CTVn_L4, *blue*; CTVn_intpect, *light green*; CTVn_IMN, *green*; CTVp_chestwall, *turquoise*; heart, *magenta*; and LADCA, *black*

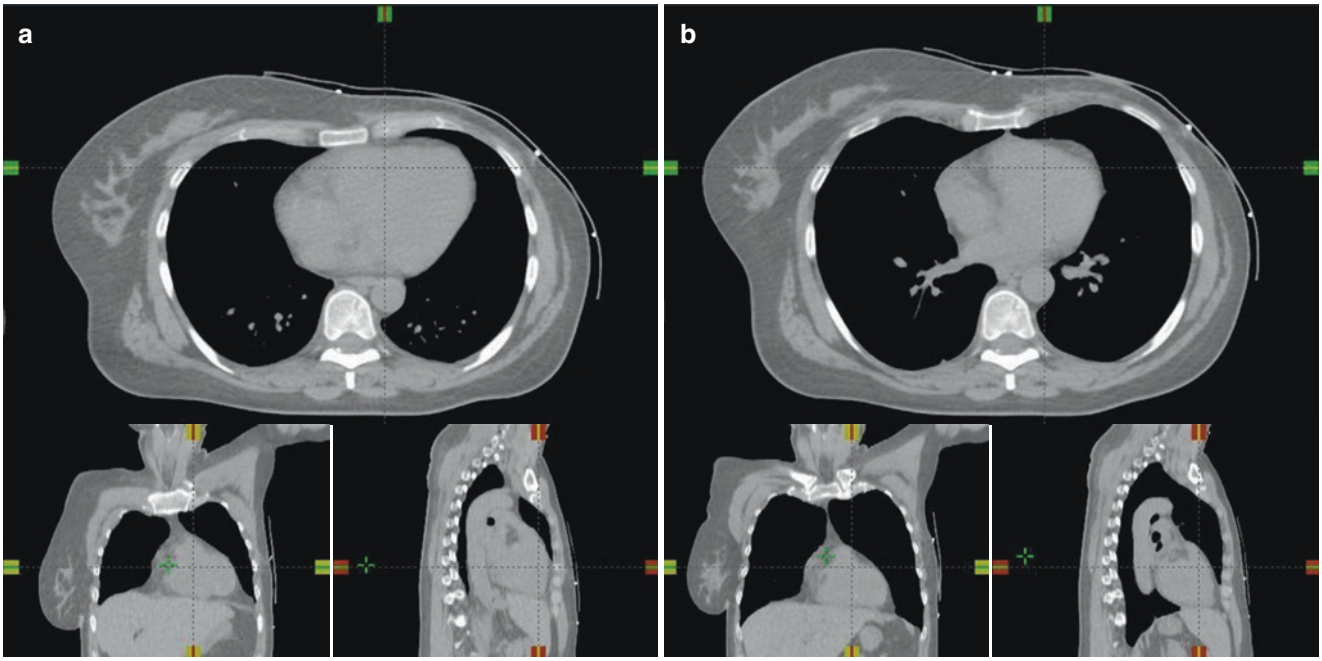

Fig. 53.2 CT scan of a patient in (**a**) free breathing and (**b**) deep inspiration breath hold

and two slightly oblique AP-PA half-beam fields covering the lymph nodes in the supraclavicular region.

One of the challenges in PMRT is to obtain a sufficient dose to the target and a minimum dose to the lung and heart. The cardiac and pulmonary complication probabilities can be reduced by using breathing-adapted RT (BART) techniques [42], and also the target coverage is improved when the patients are planned and treated in deep inspiration breath hold [43, 44]. Figure 53.2 shows the anatomical differences between free breathing and deep inspiration breath hold CT scans.

When using BART the chest wall movement is limited during treatment compared to a patient treated in free breathing. Therefore it might be expected that the setup error is smaller in BART. However, it was found that the primary benefit of BART is the separation of the heart from the target rather than a reduced setup error [45].

Apart from 3D conformal radiotherapy techniques (3DCRT) for PMRT, more advanced photon field techniques such as multiple field IMRT, helical tomotherapy and volumetric arc techniques have also been applied [46–48]. All these advanced techniques produce treatment plans which might reduce the high-dose volume in the lung and heart; however, they often increase the dose to the contralateral breast. Especially in patients younger than 40 years, it is important to limit the dose to the contralateral breast to less than 1 Gy because of an increased risk of second primary breast cancer [49]. Abo-Madyan et al. [50] have studied the risk of second cancer in the contralateral breast and both lungs after 3DCRT, IMRT and VMAT. They found that the risk of a radiation-induced second cancer was 34–50% larger after IMRT and VMAT compared to 3DCRT. The risk of inducing a second primary lung cancer after 3DCRT was determined by Grantzau et al. [51]. They found that the small absolute risk increased linearly with dose and, furthermore, that patients with a history of cigarette smoking had a significantly higher radiation-induced risk than non-smokers indicating the importance of quitting smoking before RT.

PMRT can also be carried out by using protons [52]. By using protons an appropriate chest wall and IMN coverage can be obtained with an improved cardiac sparing compared to conventional treatments by photon/electron beams. One of the challenges in proton therapy is to create a treatment plan which is robust, i.e. that the delivered dose distribution is only slightly dependent on setup uncertainties and patient breathing motion.

53.6.1 Bolus

When megavoltage photons are used, the most superficial parts of the irradiated volume will receive a lower dose due to the skin-sparing effects of this radiation. To compensate for this, a bolus of a given thickness and extension may be placed on the chest wall. For PWTF irradiation with 6 MV photons, at least 3 mm bolus is necessary to obtain an adequate dose in the PTV [53]. A survey in 2004 [54] showed significant regional differences in the use of a bolus in PMRT. Eighty-two percent of the American respondents used always a bolus, whereas the corresponding figures for

the Australasian and European respondents were 65% and 31%, respectively. There was also a wide variation in bolus thickness and in the application of the bolus every day versus on alternate days. The rationale for using a bolus in PMRT is that by increasing the dose to the skin, the risks of local recurrences are reduced. In a small study with 254 patients, Tieu et al. [55] have investigated the effect of bolus in PMRT on local recurrence. They did not find any difference between the group with a whole chest bolus compared to the group with either a parascar bolus or no bolus.

53.6.2 Breast Implants

After mastectomy a breast reconstruction with expanders and implants may be carried out. One option is to use a one-stage implant-based reconstruction with immediate implant replacement at the time of mastectomy. However, this means that if PMRT is indicated the RT will be performed on the implant. Kronowitz [56] made a literature survey showing that most of the patients undergoing implant-based reconstruction before irradiation kept the implant; however, it was also observed that irradiation resulted in a significant need for unplanned or larger corrective surgery. This may be due to radiation-induced modifications of silicone [57].

53.7 The Future of PMRT Is More Individualized Therapy

It has been established that the effect of PMRT is heterogeneous, so that in patients with ERpos/PRpos/HER2neg cancer, the 5-year gain in locoregional recurrence translates into a gain of the same magnitude at 15-year overall survival, whilst in ERneg/PRneg/HER2neg patients, the high-risk reduction in 5-year locoregional recurrence does not translate into any improved 15-year overall survival [58]. Much effort has been spent to identify a biomarker or molecular profile to help stratifying a more individualized approach to PMRT, and it was only recently that the first genetic profile predicting the benefit from PMRT was successfully identified and validated in an independent data set [59, 60]. It is to be expected that this profile and other profiles will gain more interest in the future to further individualize PMRT.

In many countries screening mammography is now standard, and it has caused a dramatic decrease in the frequency of mastectomy, because tumours today are smaller and more patients are node negative. Therefore the use of breast conservation has increased considerably. For example, in the DBCG-IMN study reporting on node-positive patients treated in the period 2003–2007, two thirds of the patients were operated with mastectomy, whilst today less than one third of new breast cancer patients are operated with mastectomy [32].

In that perspective it can be expected that the use of PMRT will decline and hopefully be prescribed in a more individualized way in the future. Based on available evidence from randomized trials, the current strategy is, however, that PMRT should be offered to patients operated with mastectomy for any node-positive breast cancer or large node-negative (pT3-4N0) breast cancer.

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Concurrent Use of Radiation Therapy and Targeted Molecules in the Breast Cancer Treatment

54

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54.1 Anti-HER2 Molecules and Radiotherapy

Several molecules can be administrated: trastuzumab, lapatinib, pertuzumab, and TDM1. The current work will present mainly the results of principal studies concerning the clinical experience of some of these molecules in association with the radiotherapy, data which could impact the everyday practice.

54.1.1 Radiotherapy and Trastuzumab

54.1.1.1 Adjuvant and Neoadjuvant Treatment

Human epidermal growth factor receptor 2 (HER2) overexpression has been observed in 20–25% of patients treated for breast cancer (BC) [1]. Amplification of the gene coding for this receptor is significantly correlated with poor prognosis in terms of local control and overall survival [2, 3].

Trastuzumab (T) (Herceptin®; Genentech, San Francisco, California) is a humanized chimeric antibody presenting antitumoral activity induced by specific binding to the extracellular domain of HER2. In the management of BC overexpressing HER2, the oncologic efficacy of T in terms of progression-free and overall survival has first been shown at a metastatic stage [4] and then in an adjuvant setting [5, 6]. However, higher incidences of cardiovascular events (CVE) have been reported in patients exposed to T [5, 6]. Significant decrease of the left ventricular ejection fraction (LVEF) and symptomatic congestive heart failure (CHF) were the most frequently observed adverse effects.

In early-stage BC, adjuvant radiotherapy (RT) improves the locoregional control as well as the overall survival compared to breast-conserving or radical surgery alone [7, 8]. However, these benefits are counterbalanced by an increased risk of cardiovascular mortality attributable to this treatment [7]. Preclinical studies have demonstrated a radiosensitizing effect of trastuzumab (T) in vitro and in vivo [9].

The concurrent administration of T with breast RT was evaluated in retrospective [10–12] and prospective clinical studies [13–15]. The objective of this review work was to prospectively assess the toxicities and the oncologic efficacy of T concurrent with adjuvant RT for nonmetastatic BC. More data is available in the association between radiotherapy and chemotherapy [16, 17].

The prospective series of the Institut Curie in 308 consecutive patients and median follow-up of 50.2 months (range: 13.0–126.0) [18] have shown the skin toxicities are close to those previously reported: 82% of acute grade 1 radiodermatitis, 13% of grade 2, 6% of late grade 1 telangiectasia, and 16% of late grade 1 skin fibrosis [13]. Some internationally accepted definitions of the irradiated volumes of the lung and heart were used in some works to obtain better assessment of the results [19, 20].

In a multicenter retrospective study on 146 patients with a median follow-up of 16 months, Belkacémi et al. [10] reported grade 2 and higher radiodermatitis in 51% of patients, occurring during or after RT. The differences compared to the results reported here can be explained by treatment in lateral position using previously published isocentric lateral decubitus (ILD), a technique developed in order to reduce skin toxicities without altering local control [21, 22]. The modalities of irradiation of the internal mammary nodes (IMN) can also contribute to a lower incidence of esophageal toxicity than that described by Belkacémi et al. [10, 23], i.e., 12% of grade 2 and higher esophagitis. Small-field electron boost to the chest wall and IMN following mastectomy reduces the photon dose [22, 24].

Shaffer et al. [11] evaluated the early cardiotoxicity of T in 59 patients; among them 44 were treated with concurrent breast RT. T was interrupted due to LVEF decrease in 6 of the patients treated by RT (13.6%) and in 5 of the 27 patients (18.5%) treated on the left breast. Three patients developed symptomatic CHF, following left breast RT. This retrospective study was mainly criticized for the heterogeneous techniques of irradiation and the short median follow-up (15 months).

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Halyard et al. [25] studied the incidence of the cardiac toxicity of T concurrent with breast RT in the patients included in the North Central Cancer Treatment Group N9831 randomized trial. The cohort was composed of 1938 patients; among them 1418 (73.2%) were treated by RT. Median follow-up was 3.7 years. The three-year cumulative incidence of CVE was 1.7% when T was initiated concurrently with paclitaxel and 2.7% in the case of a sequential treatment. No increased cardiovascular toxicity was demonstrated in patients treated by concurrent RT and T and/or by left-sided RT. Similar findings were also observed after IMN irradiation but on a limited cohort (44 patients).

The cardiotoxicity related to the treatment by T and breast RT was also studied in the patients followed in the HERA trial [26]. T was administered sequentially to the irradiation. Median follow-up was 3.6 years. According to the HERA criteria, the incidences of significant LVEF decrease and of symptomatic CHF were higher in patients treated by T than in the observation group (3.6% versus 0.6% and 1.9% versus 0.1%, respectively). A retrospective multicenter study assessed the cardiac adverse events of T administered sequentially to RT on 499 patients [27]. An asymptomatic LVEF decline strictly superior to 10% and below 20% was described in 20% of patients. The incidence of symptomatic CHF was 3%. Considering these works, the concurrent administration of T with breast RT doesn't seem to induce a significantly increased cardiotoxicity compared to a sequential treatment.

If the Institut Curie study shows limited toxicities after a median follow-up exceeding 4 years, these data have to be verified in the long term. Indeed, radiation-induced cardiac toxicity can be observed up to 20 years after the completion of the treatment [28]. Although LVEF is the parameter commonly used in daily practice, other factors have been studied to determine the risk of cardiotoxicity induced by RT. Taylor et al. [29] assessed the predictive value of the MHD to estimate the physical and biologic doses to the heart in 50 patients. They demonstrated a strong linear correlation between the MHD and the mean heart dose: for every 1 cm increase in the MHD, the mean heart dose increased by an average of 2.9% of the tumor dose [28]. In 30 patients treated for left-sided BC, Hurkmans et al. [19] highlighted a mathematical relationship between the MHD and the Normal Tissue Complication Probability (NTCP) of the heart. When the MHD was below 1 cm, the heart NTCP was estimated to be less than 1%. NTCP increased considerably to values greater than 2% when the MHD exceeded 2 cm.

Intensity modulated RT (IMRT) could improve protection of the heart during breast RT. A dosimetric study of 15 patients with MHD greater than 10 mm compared cardiac exposure to irradiation delivered by helical tomotherapy, IMRT using five to seven beams, and conformal RT [30, 31]. Mean MHD was 17 mm. Cardiac and left ventricular volumes receiving a dose of 35 Gy or more were significantly reduced using helical tomotherapy (0.5%) or IMRT (0.7%) than conformal RT (3.6%).

In conclusion, according to this review of the literature, toxicities related to the concurrent combination of T and locoregional breast RT were acceptable when adapted techniques are used. The oncologic efficacy of this treatment was also confirmed. Longer follow-up is warranted to confirm these results. The results of the studies are given in (Table 54.1).

Table 54.1 Comparison of the toxicity rates reported in patients exposed to concurrent trastuzumab with locoregional breast radiotherapy

	n	Median follow-up		Acute epithelitis (%)			Acute esophagitis (%)		Late skin reactions (%)		Late esophagitis (%)		LVEF decrease	CHF
Grade (a)			1	2	3	1	2	3	< 2	≥ 2	< 2	≥ 2		
Series														
Belkacémi et al. [10]	146	16 months	37 (b)	35	6	64 (b)	24	11	48 (c)	51	88 (c)	12	Grade 2 and greater: 10% (1) 5% (2)	–
Shaffer et al. [11]	44	15 months	–	–	–	–	–	–	–	–	–	–	Mean absolute decrease: 4% (3)	4.5%
Meattini et al. [13]	95	4.3 years	20	13.7	0	1.1	0	0	F: 18.9 T: 4.2 (d) –		–	–	Median decrease: 2% (4)	1.1%
Halyard et al. [26]	935	3.7 years	–	–	–	–	–	–	–	–	–	–	–	1.7% (i) 2.7% (ii)
Jacob et al. [18]	308	52 months	73.4 (d)	21.7	3.9	8.5 (d)	1.3	0.1	F: 18.6; T: 4.9 (e)	F:7.0; T: 3.5	0.4 (e)	0	Grade 2 and greater: 2.9% (1)	1%

CHF congestive heart failure, *F* fibrosis, *LVEF* left ventricular ejection fraction, *T* telangiectasia

(a) Skin and esophageal toxicities classification according to the Common Terminology Criteria for Adverse Events version 3.0, (b) during radiotherapy, (c) during radiotherapy or follow-up, whenever the toxicity was observed, (d) during radiotherapy or in the 6 months following the end of the irradiation, (e) more than 6 months after the completion of radiotherapy

(1) grade 2 LVEF alteration according to the Common Terminology Criteria for Adverse Events version 3.0: 40% ≤ LVEF <50%, (2) HERA criteria: LVEF diminution of 10 points or more compared to baseline assessment, or LVEF strictly below 50%, (3) considering the measurement performed before radiotherapy and the lowest value reported after the irradiation, (4) considering the baseline assessment and the LVEF observed at the last follow-up. (i) Trastuzumab administered concurrently with paclitaxel, (ii) trastuzumab delivered sequentially with paclitaxel

54.1.1.2 Concurrent Use of Radiotherapy and Trastuzumab in the Metastatic Disease

It was shown that the trastuzumab can be used concurrently with the whole brain radiotherapy in case of multiple metastases [32].

54.1.2 Double Blockade of the HER2 Receptor with the Combination of Trastuzumab and Pertuzumab and Radiotherapy

Several molecules can be administrated: trastuzumab, lapatinib, pertuzumab, and TDM1. Pertuzumab is a recombinant humanized IgG antibody which prevents the heterodimerization of HER2 receptor with HER3 which occurs after ligand binding and is a potent association for intracellular signaling [33]. Trastuzumab binds to the extracellular domain of HER2 and blocks its cleavage and the ligand-independent signaling [33]. Both can be involved in antibody-dependent cell-mediated cytotoxicity (ADCC) [34]. Double blockade of the HER2 receptor with the combination of trastuzumab and pertuzumab has a synergistic effect in vitro and in animal models [34, 35]. First studies in phase I and II with combination showed an acceptable toxicity profile [36, 37]. Digestive disorders with diarrhea can occur frequently. The main concern about toxicity is cardiac dysfunction. HER2 pathway is important for the homeostasis of cardiomyocytes [38, 39]. Left ventricular ejection fraction (LVEF) should be thoroughly controlled before anti-HER2 treatment initiation and regularly (every 3 months at least) along administration.

Baselga et al. showed in phase III an improvement in overall survival in the pertuzumab arm associated with chemotherapy with docetaxel and trastuzumab in a population of 808 patients with breast cancer presenting unresectable locoregional relapse or in first metastatic line. Progression-free survival was 18.5 months in the pertuzumab-trastuzumab-docetaxel group versus 12.4 months in the group with trastuzumab-docetaxel alone (HR: 0.62; 95% CI (0.51–0.75) $p < 0.0001$) [40]. This study named CLEOPATRA led to the approval of pertuzumab in this indication. Pertuzumab is currently evaluated in neoadjuvant and adjuvant indications in the BERENICE (NCT02132949) and APHINITY (NCT01358877) studies.

Pertuzumab has to be associated with trastuzumab during the same period of treatment, as maintenance therapy until progression in metastatic stages and for 1 year in the adjuvant setting. As a consequence, the question of the tolerance of concomitant systemic treatment with radiation therapy arises and needs to be addressed in the adjuvant context mainly but also in the metastatic setting. Adjuvant radiation therapy showed a benefit on reducing the risk of locoregional relapse and overall survival and proved itself as a standard of care [7, 8].

In controlled metastatic disease, locoregional treatment with surgery and/or radiotherapy can improve the overall survival. This was shown by Le Scodan et al. in 2008 in a population of 581 patients. Among them, 320 received a locoregional treatment consisting in exclusive radiotherapy (78%), surgery of the primary tumor followed by radiotherapy (13%), or surgery alone (9%). These patients had an overall survival at 3 years of 43.4% versus 26.7% in the group of patients who had no locoregional treatment ($p = 0.00002$). Locoregional treatment was also an independent prognostic factor (HR: 0.70; 95% CI, 0.58–0.85 $p = 0.0002$) [41]. Several other studies supported the same results [42]. Symptomatic radiotherapy is the other indication of radiation in a metastatic setting.

In vitro, a radiosensitizing effect was demonstrated in breast cancer cell lines with HER2 treatment [9]. A part from a case report [42], there are very few data on the combination of double blockade HER2 and radiation.

The series of the Institut Curie (in a population of nonselected patients) are the unique published study assessing the tolerance of the combination trastuzumab-pertuzumab and radiotherapy in patients treated for HER2+ breast cancer outside clinical trial for metastatic and/or locally recurrent unresectable disease [43]. This first exploratory study of the combination pertuzumab, trastuzumab, and radiotherapy showed in population of 23 consecutive female breast cancer patients with median age of 47 years (range: 33–85) with known cardiovascular risk factors that the observed toxicity was manageable with reversible symptoms [43]. All patients received docetaxel, pertuzumab, and trastuzumab as the first-line protocol. For docetaxel, doses were initiated at 75 mg/m^2 and then increased to 100 mg/m^2 at cycle 2 if well tolerated. For three patients, docetaxel was replaced by paclitaxel to decrease toxicity. All patients had a good partial or complete response after 6– 8 cycles enabling a maintenance protocol with pertuzumab and trastuzumab. Hormonal therapy was given concomitantly with maintenance treatment by pertuzumab and trastuzumab in six patients (26%), but only one started the hormonal therapy during the radiotherapy. Six patients (26%) previously received anthracyclines with a median cumulated total dose of 493 mg. Another potentially cardiotoxic treatment was 5FU with a median cumulated dose of 2900 mg (6pts., 26%) and history of adjuvant trastuzumab with a median cumulated dose of 6675 mg (3pts., 13%). Three patients underwent palliative radiation therapy as following antalgic bone irradiation (2 pts.) or whole brain radiotherapy (1 pt.). Most patients (20 pts., 87%) experienced locoregional radiotherapy. Notably, there was a high rate of IMN treatment with 9 pts. (39%) concerned. For 15 patients, radiotherapy was preceded by breast and lymph node surgery. Median follow-up was 7.3 months (1.2–18.9) after the start of RT and 13.8 months after the diagnosis (6.3–23.4). Observed main toxicities were five interruptions of radiotherapy (22%); of them, two were for personal issues, and one was necessary to allow urgent neurosurgery for medullary compression and in two cases for local

treatment of grade 2 or 3 radiodermatitis. Concerning skin toxicity, in 16 cases (70%) were grade 0 or 1 radiodermatitis observed. One grade 3 occurred in a 72-year-old patient treated for a contralateral relapsing inflammatory tumor of the right breast T4dNxM1 with pulmonary metastasis. Radiotherapy was performed in the right breast and the whole lymph node areas as a neoadjuvant treatment with a linear accelerator of 6MV with standard fraction of 2 Gy in ILD. A grade 3 reaction appeared at the dose of 40 Gy requiring stopping the treatment for five fractions. This patient presented two risk factors of skin toxicity with an elevated BMI of 36 and an inflammatory tumor. The observed cardiac toxicity in two patients (9%) was an asymptomatic grade 2 decrease of LVEF requiring no treatment discontinuation, and one presented a grade 3 decrease requiring a 3-month discontinuation. Neither acute cardiac failure nor symptomatic cardiac insufficiency was reported. Their common characteristics were menopausal status and age over 50 years. Two of them had a history of right-sided breast cancer and received previous radiotherapy including the IMN and anthracyclines treatment. Patients presenting a grade 2 had a decrease of LVEF of 19% and 11% compared to the initial examination. Their LVEF remained superior to 50% and they were asymptomatic. Maintenance treatment with pertuzumab and trastuzumab was continued. A part from their age (51 and 64 years) and menopausal status, these patients had no cardiovascular risk factors. One of them was treated 3 years before for a right-sided breast cancer HER2 positive and received adjuvant chemotherapy after a breast-conserving surgery with a cumulated dose of epirubicin of 480 mg and 6695 mg of trastuzumab. She underwent previous adjuvant radiotherapy in the right breast, the IMN, and the supra- and infraclavicular lymph nodes. She presented in 2014 a diffuse metastatic relapse and had a symptomatic irradiation to [T9 to L1] vertebrae at the dose of 15 Gy in five fractions of 3 Gy with a 3D conformational technique concomitantly to the treatment with P and T. The second patient had no history of early-stage breast cancer and was treated for T4Nx M1 (mediastinal and retroperitoneal lymph node metastases). This patient underwent irradiation to the chest wall and all the locoregional lymph nodes (IMN, supra- and infraclavicular lymph nodes, axilla) after a radical mastectomy. The technique used was tomotherapy: total dose was 50 Gy. The mean dose to the heart was 6.2 Gy. The patient who presented grade 3 decrease of the LVEF was a 68-year-old woman. Her cardiovascular risk factors were menopause and previous smoking. She was treated 15 years ago for cancer of the right breast T1N1 HER2−. She underwent breast-conserving surgery and adjuvant chemotherapy with 702 mg of cumulated epirubicin. She had previous adjuvant radiotherapy of the right breast, the IMN, and the supra- and infraclavicular area. She presented in 2014 with a metastatic disease to the liver, the bone, and lungs. She had a symptomatic radiation of the left scapula and the T8 to T11 vertebrae at the dose of 15 Gy in five fractions of 3 Gy concurrent to pertuzumab and trastuzumab treatment. The technique was conformational 3D. The mean dose to the heart was 4.46 Gy. The decrease of LVEF was 25%. The nadir of her LVEF was 40% and was measured over 50% 2 months later. She remained asymptomatic and had no criteria of severe cardiac event. Two patients were presented with grade 1 radiation pneumonitis diagnosed occasionally at the CT scan; they did not require any specific treatment. She underwent radiotherapy of the right breast at the dose of 50 Gy with standard fractionation. Mean dose to the lung was 9.09 Gy. Two cases of grade 1 esophagitis were reported in this study. Eighteen patients (78%) were in good partial to complete response at the time of last follow-up clinics.

In this first study of the concurrent use of radiation therapy and pertuzumab-trastuzumab association, the observed toxicity was expected, manageable, and similar to the reported association of radiotherapy-trastuzumab alone. Results of randomized trials in locally recurrent and metastatic tumors are needed with longer follow-up and prospective design to confirm our results.

Currently there is no data about the association between radiotherapy and TDM1.

54.2 Anti-angiogenic Treatment (Bevacizumab) and Radiotherapy

In the metastatic setting, there is a little data in the literature about the association of radiotherapy and bevacizumab [44, 45], especially in the treatment of brain metastases.

Following the encouraging results of bevacizumab in combination with chemotherapy in many metastatic tumor settings, bevacizumab has been developed in the adjuvant setting in breast cancer especially in the context of the BEATRICE trial. The BEATRICE trial (NCT00528567) is a phase III trial designed to evaluate the efficacy and safety of the combination of bevacizumab and chemotherapy in the adjuvant setting in patients with triple-negative breast cancer [46]. Patients included in the trial were randomized to either the standard treatment arm comprising standard adjuvant chemotherapy alone or followed by radiotherapy according to the standard practice of each center or the experimental arm comprising standard chemotherapy combined with bevacizumab either alone or followed by radiotherapy. BEATRICE randomization was stratified according to type of surgery, lymph node status, chemotherapy, and hormone receptor status. The dose of bevacizumab administered had to correspond to the equivalent of 5 mg/kg per week, and the duration of bevacizumab therapy was 1 year. The primary objective of this study was to determine the disease-free survival in each of the two arms. Patient inclusion has ended and the trial is currently underway. During this trial, patients included in the experimental arm received bevacizumab concurrently with local ± regional

radiotherapy of the breast. However, the long-term effects of this concurrent combination on the skin, healthy breast tissues, lung, and heart included in breast and lymph node irradiation volumes are unknown. The only available data are derived from the retrospective comparative study by Goyal and colleagues based on 14 patients who received concurrent bevacizumab and radiotherapy and matched with controls [47]. The results of this study demonstrated the good safety of concurrent bevacizumab and radiotherapy with no acute locoregional toxicity ≥ grade 3 according to the CTCAE v3 scale. However, the study by Goyal and colleagues only reported the acute toxicities of the combination and no data on late toxicities are available at the present time.

The objectives of the French multicenter TOLERAB study [48, 49] were to evaluate, in the cohort of patients included in the BEATRICE trial treated by concurrent bevacizumab and radiotherapy and in the cohort of patients treated by radiotherapy alone, (1) acute locoregional toxicity, (2) the cosmetic result for patients managed by breast-conserving therapy, and (3) late locoregional toxicity. Eighty-four patients who had participated in the BEATRICE trial were included in this French multicenter cohort study between October 2007 and November 2012. The cohort without bevacizumab and the cohort with bevacizumab comprised 45 patients and 39 patients, respectively. Evaluation 1 year after completion of radiotherapy was available for all patients, and acute and late locoregional toxicities of radiotherapy were able to be compared between the 45 patients who received adjuvant radiotherapy alone and the 39 patients who received the concurrent bevacizumab and radiotherapy combination.

The baseline evaluation is comprised of recording of medical history, WHO performance status, physical examination, and a laboratory work-up. Patients were evaluated once a week during radiotherapy, 4 weeks after completion of radiotherapy, and then every 3–6 months thereafter. Data concerning the locoregional toxicity of treatment included acute radiation dermatitis, acute esophagitis, pain, and radiation fibrosis, lymphedema of the arm, ulcerations, telangiectasia, dysphagia, dyspnea, plexitis, pericarditis, and myocardial infarction. Acute toxicity was graded according to the Common Toxicity Criteria for Adverse Events version 3 or CTCAE v3. According to this system, acute toxicity is graded from grade 1 (minor toxicity) to grade 5 (death). In this study, acute toxicities were considered to be severe for grades greater than or equal to 3. In the bevacizumab arm, 67% of patients were postmenopausal. The majority of patients (62%) were between the ages of 40 and 60 years. The left breast was affected in 56% of cases. Clinical stages of breast cancer were distributed as follows: 49% stage I and 51% stage II. The most common histological type was invasive ductal carcinoma (87% of cases), histological grade was generally high (grade 3 in 85% of cases), and all patients had triple-negative breast cancer. Among the 39 patients who received concurrent bevacizumab and radiotherapy, 35 (90%) received radiotherapy to the breast alone at a median dose of 50 Gy plus a boost to the tumor bed at a median dose of 16 Gy, and 4 patients (10%) received chest wall radiotherapy at a median dose of 50 Gy. Radiotherapy of the draining lymph nodes was performed in 19 patients (49%); this radiotherapy concerned the supraclavicular nodes in 44% of cases and the internal mammary nodes in 31% of cases. The median duration of radiotherapy was 49 days for breast radiotherapy with a boost to the tumor bed ± lymph nodes and 38 days for radiotherapy of the chest wall ± lymph nodes. The median interval between surgery and radiotherapy was 184 days. Among the 45 patients who did not receive concurrent bevacizumab with radiotherapy, 38 (84%) received radiotherapy to the breast alone at a median dose of 50 Gy plus a boost to the tumor bed at a median dose of 16 Gy, and 7 patients (16%) received chest wall radiotherapy at a median dose of 50 Gy. Radiotherapy of the draining lymph nodes was performed in 21 patients (47%); this radiotherapy concerned the supraclavicular nodes in 44% of cases and the internal mammary nodes in 31% of cases. The median duration of radiotherapy was 49 days for breast radiotherapy with a boost to the tumor bed ± lymph nodes and 38 days for radiotherapy of the chest wall ± lymph nodes. The median interval between surgery and radiotherapy was 186 days. No significant difference was observed between the two arms [49]. The early and late results of the study are given in Table 54.2. Late toxicities at 1 year after the completion of radiotherapy were available for 38 patients (97%) in the bevacizumab arm and 40 patients (89%) in the arm without bevacizumab, respectively. Grade 1–2 toxicities reported in the bevacizumab arm were pain in seven patients (18%), fibrosis in three patients (8%), telangiectasia in two patients (5%), and dyspnea in one patient (3%). No grade 3–4 toxicity was observed. Grade 1–2 toxicities reported in the arm without concurrent bevacizumab were pain in six patients (15%), fibrosis in two patients (5%), lymphedema in two patients (5%), and paresis in two patients (5%). No grade 3–4 toxicity was observed. In this work studying population of the BEATRICE randomized trial, we compared the acute and late toxicity rates between the experimental arm comprising concurrent bevacizumab (39 patients) and the standard arm without bevacizumab (45 patients). A low rate of toxicity was observed in both arms, and no significant difference was observed between the two arms in terms of acute toxicities, cosmetic results, and late toxicities. These results concerning acute toxicity are therefore comparable to those reported by Goyal and colleagues while also providing an analysis of esophageal toxicity, cosmetic effects, and late toxicity [47]. Currently, the evaluation of the results at 3 and 5 years is running [48, 49]. These results of this multicenter study with 1-year follow-up indicate the acceptable toxicity of concurrent bevacizumab and locoregional radiotherapy for breast cancer. Nevertheless, these results need to be confirmed with longer follow-up. However, continuation of concurrent bevacizumab and radiotherapy cannot be recommended for metastatic breast cancer in the absence of data beyond 1 year.

Table 54.2 Acute toxicities and cosmetic results, late toxicities (Tolerab study)

Acute dermatitis		Bevacizumab + RT *N* (%)		RT alone		*p*-Value
Acute dermatitis evaluation	Yes No	35 (90%) 4 (10%)		41 (91%) 4 (9%)		NS[a]
Grade	Grade 0 Grade 1 Grade 2 Grade 3 Grade 4	5 (14%) 18 (51%) 9 (26%) 3 (9%) 0		7 (17%) 24 (59%) 8 (19%) 2 (5%) 0		NS
Acute esophagitis evaluation	Yes No	39 (100%) 0 (0%)		44 (98%) 1 (2%)		NS
Grade	Grade 0 Grade 1 Grade 2 Grade 3 Grade 4	38 (97%) 0 1 (3%) 0 0		44 (100%) 0 0 0 0		NS
Cosmetic results		Bevacizumab + RT *N* (%)		RT alone *N* (%)		*p*-Value
Cosmetic evaluation	Yes No	25 (64%) 14 (36%)		26 (58%) 19 (42%)		NS[a]
Cosmetic results	Grade 0 (no change) Grade 1 (minor changes) Grade 2 (operated breast deformed) Grade 3	13 (33%) 9 (23%) 3 (8%) 0		16 (36%) 10 (22%) 0 0		NS
Late toxicities		Bevacizumab + RT *N* (%)		RT alone *N* (%)		
Late toxicities evaluation	Yes No	38 (97%) 1 (3%)		40 (89%) 5 (11%)		
		Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	
Pain		7 (18%)	0	6 (15%)	0	
Fibrosis		3 (8%)	0	2 (5%)	0	
Telangiectasia		2 (5%)	0	0	0	
Arm lymphedema		3 (8%)	0	2 (5%)	0	
Ulceration		0	0	0	0	
Myocardial ischemia		0	0	0	0	
Pericarditis		0	0	0	0	
Dyspnea		1 (3%)	0	0	0	
Dysphagia		0	0	0	0	
Paresis		0	0	2 (5%)	0	

[a]*NS* not significant

54.3 New Directions: Targeted Therapy with Olaparib (PARP Inhibitor) and Radiotherapy

54.3.1 For BRCA Mutation-Positive Breast Cancer

PARP inhibition is a novel approach to targeting tumors with deficiencies in DNA repair mechanisms. PARP enzymes are essential for repairing DNA single-strand breaks (SSBs). Inhibiting PARPs leads to the persistence of SSBs, which are then converted to the more serious DNA double-strand breaks (DSBs) during the process of DNA replication. During the process of cell division, DSBs can be efficiently repaired in normal cells by homologous recombination repair (HR). Tumors with HR deficiencies (HRD), such as serous ovarian cancers and breast cancer, cannot accurately repair the DNA damage, which may become lethal to cells as it accumulates. In such tumor types, olaparib may offer a potentially efficacious and less toxic cancer treatment compared with currently available chemotherapy regimens. Olaparib (AZD2281, KU-0059436) is a potent polyadenosine 5′diphosphoribose [poly(ADP-ribose)] polymerization (PARP) inhibitor (PARP-1, PARP-2, and PARP-3) that is being developed as an oral therapy, both as a monotherapy (including maintenance) and for combination with chemotherapy and other anticancer agents. Olaparib has been shown to inhibit selected tumor cell lines in vitro and in xenograft and primary explant models as well as in genetic BRCA knockout

models, either as a stand-alone treatment or in combination with established chemotherapies. Cells deficient in homologous recombination DNA repair factors, notably BRCA1/2, are particularly sensitive to olaparib treatment. Cellular DNA is continually subject to damage, which coordinated pathways act to repair, thereby maintaining genomic integrity and cell survival [50–52]. The poly(adenosine diphosphate [ADP]-ribose) polymerases (PARPs) are a large family of multifunctional enzymes, the most abundant of which is PARP-1. It plays a key role in the repair of DNA single-strand breaks through the repair of base excisions [53, 54]. The inhibition of PARPs leads to the accumulation of DNA single-strand breaks, which can lead to DNA double-strand breaks at replication forks. Normally, these breaks are repaired by means of the error-free homologous recombination double-stranded DNA repair pathway, key components of which are the tumor-suppressor proteins BRCA1 and BRCA2. PARP inhibitors such as olaparib show promising results in clinical trials, especially in triple-negative breast (or ovarian) cancer with *BRCA* mutations [53].

54.3.2 PARP Inhibitors Activity in Triple-Negative Breast Cancer (TNBC) Without BRCA Mutation

The challenges of TNBC are in fact more fundamental than insensitivity to current available therapeutics. TNBC shares clinical and pathological features with hereditary BRCA1-related breast cancers and in sporadic TNBC; deregulation of *BRCA1* has been frequently observed together with other defects in homologous recombination pathways [54]. Preclinical studies have shown that breast cancer cell lines with a triple-negative phenotype are more sensitive to PARP1 inhibitors compared with non-TNBC cells and that PARP inhibition synergizes with gemcitabine and cisplatin in triple-negative cells but not in luminal cancers [55]. All these lines of evidence provide a strong rationale for developing a new therapeutic approach to TNBC based on targeting the DNA repair defects via PARP inhibition in these cancers that the most aggressive are the inflammatory, locoregional advanced, and metastatic breast cancer.

54.3.3 Mechanisms of Radiosensitization by Olaparib

- *Molecular mechanism—increase of double-strand breaks*:
  DNA is the principal target for the biologic effects of radiation. For radiotherapy, this comprises single-strand breaks (SSBs) and double-strand breaks (DSBs). SSBs are not directly cytotoxic but during DNA replication may generate potentially lethal DSB by collapse of stalled replication forks [56]. Radiation-induced SSBs are primarily repaired by base excision repair, of which poly(ADP-ribose) polymerase-1 (PARP-1) is a key component. PARP-1 binds to SSB, activating poly ADP-ribosylation of itself and other proteins, triggering recruitment of repair factors and release of PARP-1 from the damaged site. PARP inhibitors inhibit SSB repair, and the unrepaired SSBs generate collapsed replication forks which give rise to potentially lethal DSB, leading to radiosensitization [57].
- *Cellular mechanism—radiosensitization during the S phase*:
  Experiments using synchronized HeLa cells showed that radiosensitization induced by PARP inhibition is specific of the S phase of the cell cycle and involves stalled replication forks [58]. Under these conditions, prolonged contact with ANI ended in the formation of de novo DNA double-strand breaks hours after irradiation, evoking collision with uncontrolled replication forks of DNA lesions whose repair was impaired by inhibition of the PARP catalytic activity. The data suggest that increased response to radiotherapy by PARP inhibitors may be achieved only in rapidly growing tumors with a high S-phase content.
- *Tissular mechanism—vasoactive effects contributing to tumor reoxygenation*:
  Recently, at least two new generation PARP inhibitors (AG014699 and AG14361) have been reported to have vasoactive properties, and AG14361 has been shown to enhance the response to radiation [59, 60]. The new generation PARP inhibitors, including olaparib, are all structurally related to nicotinamide which can prevent intermittent vascular shutdown in tumors. Senra et al. showed in preclinical studies that olaparib is a more potent vasorelaxant than nicotinamide, and its effects are maintained during treatment with drug alone and when drug and radiation are combined in a fractionated treatment schedule [61].

54.3.4 Results of In Vivo Studies of Olaparib with Concurrent Radiotherapy in Triple-Negative Breast Cancer Xenograft

Results from Inserm U612 have shown that olaparib radiosensitizes TNBC models [62]. The BRCA2−/− HBCx-17 and the wild-type HBCx-12A xenografts were subcutaneously transplanted into the flanks of nude mice. In both TNBC models, individual group comparisons showed that treatment with 4-[(3-[(4-cyclopropylcarbonyl) piperazin-4-yl] carbonyl)-4-fluorophenyl] methyl (2H) phthalazin-1 alone for 4 weeks markedly inhibited tumor growth compared with the untreated controls. Treatment with radiotherapy alone also resulted in significant growth inhibition, whereas combination of 4-[(3-[(4-cyclopropylcarbonyl) piperazin-4-yl] carbonyl)-4-fluorophenyl] methyl (2H) phthalazin-1 and radiotherapy showed the best treatment response.

The association between radiation therapy and systemic treatment can be an interesting treatment option in cases with refractory and rapidly progressive disease [63].

These results have shown that this association could be an interesting therapeutic option in the breast cancer and currently two phase I studies are running in Europe: in the Institut Curie for locally advanced, metastatic TNBC and in NKI Amsterdam for nonoperable BC. These studies will be followed with great interest.

As general conclusion, we can add that new targeted treatments are coming rapidly in the treatment of breast cancer, and it is urgent to evaluate the efficacy and toxicity of their association with the radiotherapy in the clinical studies. Highly performing radiotherapy techniques must be used. Parallel biological studies are needed to find the predictors of the tumor responses.

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Accelerated Partial Breast Irradiation

55

Nina N. Sanford and Alphonse G. Taghian

55.1 Rationale

Multiple randomized studies have conclusively demonstrated that adjuvant radiotherapy improves local control in patients with early-stage breast cancer who are treated with breast-conserving surgery (i.e., lumpectomy) [1, 2]. Furthermore, two subsequent trials assessing the additional benefit of adding a boost to the tumor bed showed a statistically significant reduction in ipsilateral breast tumor recurrence with greater absolute benefit in patients of younger age [3, 4]. On the basis of these randomized studies, whole breast radiation therapy over 5 weeks followed by a boost over 1 week has become a standard of care in many countries across the world.

Nevertheless, despite the many advantages of this treatment paradigm, there are also notable disadvantages to a 6-week course of adjuvant radiotherapy, notably inconvenience and cost. As a result, a proportion of patients who have undergone lumpectomy elect to forgo adjuvant radiotherapy, which can have serious consequences on their cancer-specific survival and overall survival. Two US studies showed that between 14 and 20% of women do not receive radiotherapy after undergoing breast-conserving surgery [5, 6]. In countries with scarce radiation availability, even for patients who wish to undergo radiotherapy, such a prolonged treatment course can lead to delays in initiating treatment.

One approach to overcome these concerns is to shorten the course of radiotherapy. Two large randomized trials assessing the outcome of hypofractionated whole breast radiotherapy over 15 or 16 fractions have demonstrated similar local regional relapse rates and cosmetic outcomes as compared to standard fractionation over 25 treatments [7, 8]. However, similar to whole breast radiotherapy, hypofractionated irradiation treats the entire breast resulting in unnecessary radiation to nontarget breast tissue in selected patients, as well as exposure to surrounding normal structures including the heart and lung. In addition, with the inclusion of a tumor bed boost, this treatment schedule still requires approximately 1 month of daily radiotherapy.

Accelerated partial breast irradiation (APBI) drastically shortens the radiation treatment schedule to 1 week or less by further increasing the dose per fraction. In order to safely deliver a high daily radiation dose and minimize normal tissue injury, the volume treated is reduced to the tumor bed plus a margin. The rationale for directing radiation to the tumor bed is that in patients who have undergone lumpectomy, greater than 80% of recurrences will involve the site of original disease [2, 9]. Thus, the hypothesis behind APBI is that by increasing the dose per fraction to condense treatment while reducing the irradiated volume, an excellent therapeutic ratio can be achieved. In addition to improving patient convenience and access, other potential advantages of APBI include (1) reduction in acute and long-term toxicities due to less radiation dose exposure by normal tissues (i.e., radiation pneumonitis, coronary artery disease for left-sided cancers), (2) reduction in overall treatment expenditure, and (3) eligibility in select patients who recur locally to undergo a second course of breast conservation therapy with re-irradiation (as opposed to recurrence after whole breast irradiation where mastectomy is often the only local treatment choice).

55.2 History

The first trials assessing APBI were conducted more than 20 years ago in the United Kingdom. In a large study involving 708 patients who underwent lumpectomy and were randomized to whole breast with regional nodal irradiation versus fractionated external beam electron radiotherapy to the tumor bed alone using a direct electron field, the 8-year local recurrence rate was 25% for patients treated with partial breast irradiation and 13% for patients receiving whole breast irradiation

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[10]. In addition, cosmetic outcomes were worse in the APBI group with higher rates of fibrosis and telangiectasias. In a second smaller study, 27 patients who underwent breast-conserving surgery received an iridium-192 brachytherapy implant that delivered 55 Gy on a continuous basis over 5 days [11]. At a median follow-up of 6 years, local relapse was 37%. As a result of the high local recurrence rates, further research on APBI was abandoned for the next few years.

Beginning in the late 1990s, there was renewed interest in APBI with the hope that due to advancements in imaging systems, pathologic analysis, radiation treatment planning, and more rigorous patient selection, the outcomes of APBI could be significantly improved upon. These modern APBI techniques and studies will be described in further detail in subsequent sections of this chapter.

55.3 Radiobiology

Historically, assumptions regarding tumor and normal tissue sensitivity to fraction size have been derived from data on squamous cell cancers. An α-β ratio of 3 is assumed for most normal tissues, while the majority of tumors are thought to have an α-β ratio of 10. Suggestion that breast tumors may have a lower α-β ratio thus making them more sensitive to fraction size stems from the UK START A trial, which randomized women into three fractionation schedules that are isoeffective when α-β ratios of 6 and 1.8 were assumed for breast tumor and normal tissues, respectively. The three treatment regimens were 50 Gy in 25 fractions (control arm), 39 Gy in 13 fractions, and 41.6 Gy in 13 fractions, all delivered over 5 weeks. After a median follow-up of 9.3 years, there was no statistically significant difference in the rate of local-regional relapse between the 41.6 Gy and 50 Gy arms or the 39 Gy and 50 Gy arms. Normal tissue toxicities including moderate or marked breast induration, telangiectasias, and edema were less common in the 39 Gy group than in the 50 Gy group. A meta-analysis of the START A and the START pilot trial provided an adjusted α-β ratio of 3.5 [7]. The α-β ratio for late-responding breast tissues ranged from 3.5 for breast shrinkage to 4.7 for breast edema. Similarly, the Canadian hypofractionation trial randomized women to either 50 Gy in 25 fractions or 42.5 Gy in 16 fractions and also found comparable local control in both arms, suggesting a lower α-β ratio (between 3 and 4) for breast tumors [8]. There was also suggestion that hypofractionation in this study was associated with decreased acute toxicity and improved quality of life attributed to decreased skin and breast side effects, decreased fatigue, and improved convenience [12]. A recent randomized trial including 287 women comparing conventionally fractionated versus hypofractionated whole breast radiotherapy also found that rates of acute toxicity, fatigue during treatment, and at 6-month follow-up were statistically significantly lower in the hypofractionated arm [13].

Table 55.1 Comparison of BED values for various dose fractionation schedules

Protocol schedule	Tumor control (α-β = 4 Gy)	Tumor control (α-β = 10 Gy)
Standard		
2 Gy × 25	75	60
2 Gy × 30	90	72
2 Gy × 33	99	79
APBI		
3.85 Gy × 10	76	53
3.4 Gy × 10	63	46

BED biological effective dose

With a lower tumor α-β ratio, small increases in fraction size can produce significant changes in radiotherapy effect. A comparison of BED values for three standard whole breast irradiation protocols and 12 different hypofractionated APBI regimens found that assuming an α-β ratio of 10, the BED for tumor control was higher with standard fractionation [14]. However, using an α-β ratio of 4, the BED values of most APBI protocols resulted in tumor control BEDs closer to 50 Gy standard treatment, although lower than BEDs for regimens treating to a total of 60Gy or 66Gy (Table 55.1).

However, the BED equation does not take into account treatment frequency. It is hypothesized that accelerated therapies may prevent tumor proliferation and repopulation during therapy, suggesting another advantage to APBI. It is also important to note however that these calculations are based off of imperfect radiobiological modeling systems and that the BED equation, in particular, is thought to be less valuable at higher doses per fraction. The true efficacy and safety of APBI can therefore only be demonstrated through large, well-designed, patient studies.

55.4 Patient Eligibility

Appropriate patient selection is critical in determining the success of APBI. Outside of the clinical trial setting, APBI is currently restricted to patients with the low risk disease. Patient and disease characteristics often used to categorize an individual as low risk include older age, small tumor size, no extensive intraductal component (EIC), estrogen receptor (ER) positive, and node negative. These patients are felt to have some risk of harboring residual disease in proximity to the tumor bed but very little possibility of harboring residual microscopic disease in remote locations in the ipsilateral breast or lymph nodes. There is no uniform consensus on which patients are appropriate for APBI with ongoing trials seeking to better define and potentially broaden eligibility. Shown in Table 55.2 are criteria from four large organizations including ASTRO, European, American Brachytherapy Society, and American Society of Breast Surgeons. In later sections of this chapter, we contrast these guidelines to the eligibility criteria

Table 55.2 Eligibility criteria for APBI

	ASTRO [15][a]			GEC-ESTRO [16]			ASBS [17]		ABS [18]
Factor	Suitable	Cautionary	Unsuitable	Low-risk/good candidate	Intermediate-risk/ possible candidate	High-risk/ contraindicated	Suitable	Unsuitable	Acceptable
Age (years)	≥50	40–49[c] ≥50[d]	<40 40–49[e]	>50	>40–50	≤40	≥45 if invasive carcinoma, ≥50 if DCIS	<45 if invasive carcinoma or LCIS, <50 if DCIS	≥50
Size (invasive disease)	≤2 cm	2.1–3.0 cm	>3 cm	≤3 cm	≤3 cm	>3 cm	≤3 cm		≤3 cm
T stage	Tis[b] or T1	T2	T3/T4	T1/T2	T1/T2	T2 (if >3 cm), T3, T4	T0 (≤3 cm), T1, T2 (≤3 cm)	T0 (>3 cm), T2 (>3 cm), T3-T4	T1, T2 (≤3 cm)
N stage	N0	N/A	N1[a]	N0	pN1mic-N1a	pN2a[a]	N0	≥N1	N0
Multicentricity and multifocality	Unicentric and unifocal	N/A	Multicentric or multifocal	Unicentric and unifocal	Unicentric and multifocal (limited to within 2 cm of index lesion)	Multicentric or multifocal (>2 cm from the index lesion)			
BRCA 1/2 mutation	Not present	N/A	Present						
Grade	Any	N/A	N/A	Any	N/A				
LVSI	No	Limited/focal	Extensive	No	No	Present			No
ER	Positive	Negative	N/A	Any	N/A	N/A			
Nodal surgery	SLNB or ALND	N/A	Not performed	SLNB or ALND	ALND (or at least 6 LN examined)				
Margins	Negative (≥2 mm)	Close (<2 mm)	Positive	Negative (≥2 mm)	Close (<2 mm)	Positive	Negative	Positive	Negative
Histology	IDC and other favorable, DCIS[b]	DCIS (≤3 cm) or ILC	DCIS (≥3 cm)	IDC and other favorable histologies	IDC and other favorable histologies or ILC		Invasive carcinoma or DCIS		Invasive carcinoma or DCIS
EIC	None	Present and tumor size ≤3 cm	Present and tumor size >3 cm	None	None	Present			
Associated LCIS	Allowed	N/A	N/A	Allowed	N/A	N/A			
Neoadjuvant chemotherapy	Not used	N/A	Used	Not used	No	Used			

ASTRO American Society for Radiation Oncology, *GEC-ESTRO* The Groupe Européen de Curiethérapie (GEC), *ESTRO* the European Society for Radiotherapy and Oncology, *ASBS*, American Society of Breast Surgeons, *ABS* American Brachytherapy Society, *IDC* invasive ductal carcinoma; *ILC* invasive lobular carcinoma, *DCIS* ductal carcinoma in situ, *ALND* axillary lymph node dissection, *SLNB* sentinel lymph node biopsy

[a]Updated from 2009 guidelines

[b]DCIS allowed if: screen-detected, low to intermediate nuclear grade, size ≤2.5 cm and resected with margins negative at ≥3 mm

[c]If all other criteria for "suitable" are met

[d]If patient has at least 1 of the pathologic factors in the "cautionary" group and does not have any "unsuitable" factors

[e]If patient does not meet criteria for cautionary

of randomized Phase III trials, including the NSABP B39/RTOG 0413 trial, which are generally more liberal, thus allowing the inclusion of higher-risk patients.

55.5 APBI Techniques

Although all APBI treatments share the singular aim of condensing breast radiotherapy to 1 week or less by increasing the dose per fraction of radiation while decreasing the target volume, there are multiple approaches to accomplishing this goal. These methods differ in several ways including treating time, radiation dose, and, perhaps most importantly, in the volume of breast tissue irradiated. Each has distinct advantages and challenges. Often, more than one approach can be successfully employed, and the treatment of choice depends on technical availability and physician and patient preference. Currently, the four principle methods of APBI include (1) multicatheter interstitial brachytherapy, (2) balloon-based brachytherapy (MammoSite), (3) external beam radiotherapy, and (4) intraoperative radiotherapy.

55.5.1 Multicatheter Interstitial Brachytherapy

Multicatheter interstitial brachytherapy (MIB) was the first APBI technique developed and, as a result, has generated the longest follow-up data at this time. Initially, MIB was performed at the time of lumpectomy and used as a boost prior to standard whole breast irradiation. Over the subsequent decades, the technique has evolved to its current indication as the sole radiation treatment after lumpectomy.

The general strategy of MIB is to place under anesthesia multiple needles or tubes across the tumor bed. The specific catheter orientation is individualized for each tumor to use as few catheters as possible in an arrangement that is comfortable for the patient while adequately dosing the tumor. Generally, 15–25 catheters are required per patient. Image guidance, which can be achieved with ultrasound, stereotactic mammogram, or, more commonly, CT, is performed at several stages including before catheter insertion to select the optimal approach, at periodic intervals during insertion to confirm placement, and at the end of insertion to transfer to brachytherapy planning software. During treatment planning, the target volume, which is usually the tumor cavity plus a 1–2 cm margin, is delineated so that the optimal dwell times can be determined. General dosimetric goals include ensuring that at least 90% of the target receives 90% of the prescribed dose while preventing excess dose inhomogeneity by limiting the volume of breast tissue (target or nontarget) receiving 200% and 150% of the prescribed dose [19–21].

The dose can be delivered using continuous low-dose rate (LDR) brachytherapy, usually with iodine-125, or with high-dose rate (HDR) brachytherapy. HDR brachytherapy is now more frequently employed because it allows for better control of dosimetry and permits delivery of treatment on an outpatient basis. One HDR fractionation and source that is commonly used is 34 Gy in 3.4 Gy BID fractions with iridium-192. All treatment catheters remain in the patient's breast for the entire duration of the treatment course. As opposed to MammoSite and 3DCRT, MIB can be used in almost all cavity sizes, shapes, and locations within the breast, and dose distribution can be individually tailored to minimize hotspots (Fig. 55.1). Disadvantages however

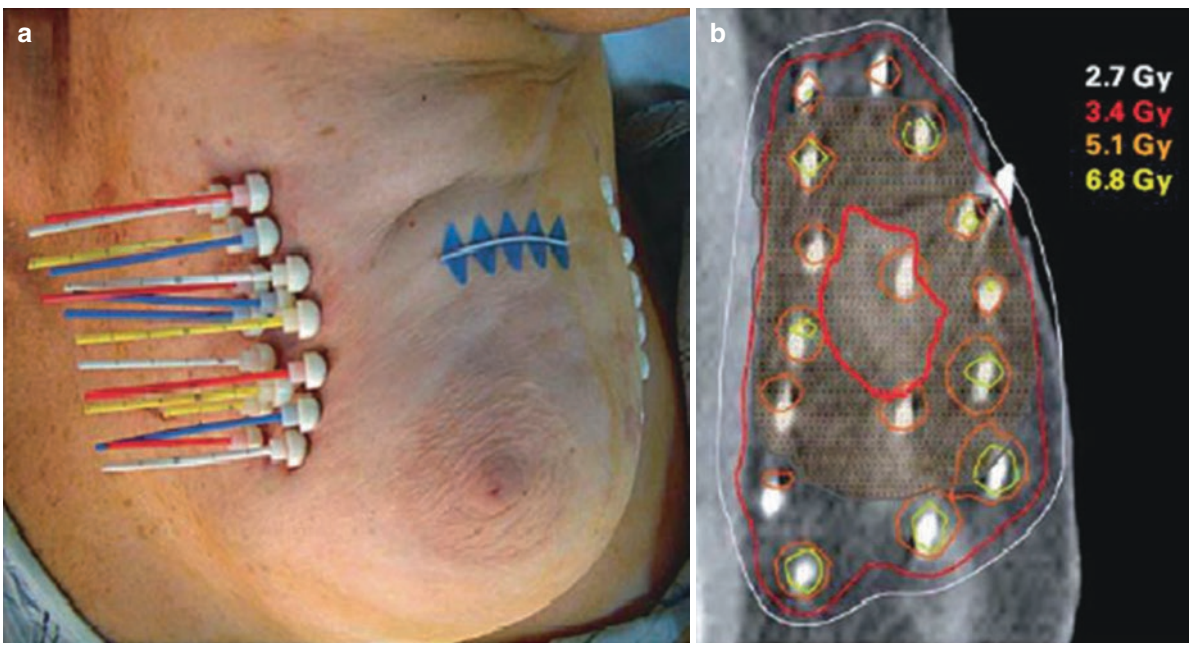

Fig. 55.1 Multicatheter interstitial brachytherapy showing catheter placement externally and internally with dosimetric coverage. The lumpectomy cavity is outlined in *red* and target volume in *orange* [22]

Table 55.3 Results of trials on multicatheter interstitial brachytherapy APBI

Institution	# of patients	Follow-up (months)	Local recurrence	% Good/excellent cosmesis
NIO-Hungary (Phase II) [23]	45	136	13.8% (15 years)	78%
RTOG 95-17 (ASCO 2012)	99	146	6.2% (10 years)	68%
William Beaumont Hospital [24]	199	113	5% (12 years)	NR
Orebro University [25]	50	86	4% (7 years)	56%
MGH [26]	48	134	15% (12 years)	67%
Tufts/Brown University [27]	33	84	6.1% (5 years)	88%
Ochsner Clinic [28]	50	74	2% (crude)	75%
German-Austrian MC Trial [29]	274	63	2% (5 years)	90%
University of Wisconsin [30]	136 (cautionary)	60	4.8% (crude)	NR
Washington University [31]	202	60	3% (5 years)	NR
VCU [19]	44	44	0% (4 years)	90
University of Kansas [32]	24	47	0% (4 years)	NR
University of Perugia, Italy [33]	80	30	0% (30 months)	99%

NIO National Institute of Oncology, *RTOG* Radiation Therapy Oncology Group, *MGH* Massachusetts General Hospital, *VCU* Virginia Commonwealth University

include requiring specialized training with results that are highly operator dependent. In addition, the procedure is resource intensive and requires anesthesia support and occasionally time in the operating room.

In reviewing the literature on MIB summarized in Table 55.3, it is important to recognize that there are significant discrepancies in treatment techniques which have evolved considerably over time. In addition, breast imaging and pathologic analysis have also improved; thus, comparisons of study outcomes are likely not valid. Furthermore, most of the published data are from single institution Phase I/II studies.

55.5.2 MammoSite

The MammoSite applicator, another form of brachytherapy, was initially developed in the early 2000s with the goal of simplifying partial breast brachytherapy, thereby making it more accessible and reproducible. It utilizes an HDR source, usually iridium-192, at the center of an inflatable balloon applicator that is placed inside the surgical cavity following breast-conserving surgery. The device is inflated to fill the entire tumor bed and deliver a high dose of radiation that rapidly falls off covering approximately 1 cm of the surrounding breast tissue. Conformality, which describes the fit of the balloon inside of the cavity, is closely related to the degree of target coverage (Fig. 55.2). The recommended minimal acceptable coverage is D90 of 90%.

Advantages of MammoSite over multicatheter interstitial brachytherapy include that it is relatively easier to use, the dosimetry is simpler, and the insertion process is less traumatic for the patient. This simplicity however also leads to several drawbacks. Notably, the premade single catheter balloons cannot be customized for irregularly shaped surgical cavities or those too close to the chest wall or skin surface. The dosimetry in these circumstances may be unacceptable, precluding a small proportion of patients from treatment with MammoSite. This limitation has been partially circumvented via the design of elliptical shaped balloons and the availability of multiple dwell catheters.

The initial MammoSite study included 70 patients in a prospective Phase I/II trial [34]. Eligibility criteria included age ≥45 years, tumor size ≤2 cm but with post-lumpectomy cavity ≥3 cm, invasive ductal histology, negative lymph nodes, and negative margins. Patients with extensive intraductal component (EIC), with lobular histology, or with underlying collagen vascular disease were excluded. Patients were also ineligible if they were found to have cavities that were too large (acceptable diameters were 4–5 cm), had poor balloon-cavity conformance, or inadequate balloon-skin distance, all factors that would lead to unfavorable dosimetry. These anatomic variables were assessed using CT imaging after device placement. A dose of 34 Gy in 3.4 Gy twice daily fractions was prescribed to a point 1 cm from the balloon surface which corresponds to a surface dose of 225% for a 4 cm balloon. The procedure could be performed at the time of lumpectomy for patients enrolled preoperatively and up to 10 weeks after surgery. Of the 70 patients enrolled, 43 were ultimately treated with MammoSite brachytherapy. The treatment was well tolerated with the most common side effects associated with placement including mild erythema, drainage, pain, and ecchymosis. Toxicities during radiotherapy were similar and also included mild erythema and pain along with dry desquamation. At 1 month, 88% of patients had good-to-excellent cosmetic outcome. There was an association between skin spacing, defined as the distance between the balloon and the skin, and cosmesis: patients with skin spacing of 5–7 mm had higher rates of telangiectasias than those with spacing ≥7 mm (67% vs. 29%,

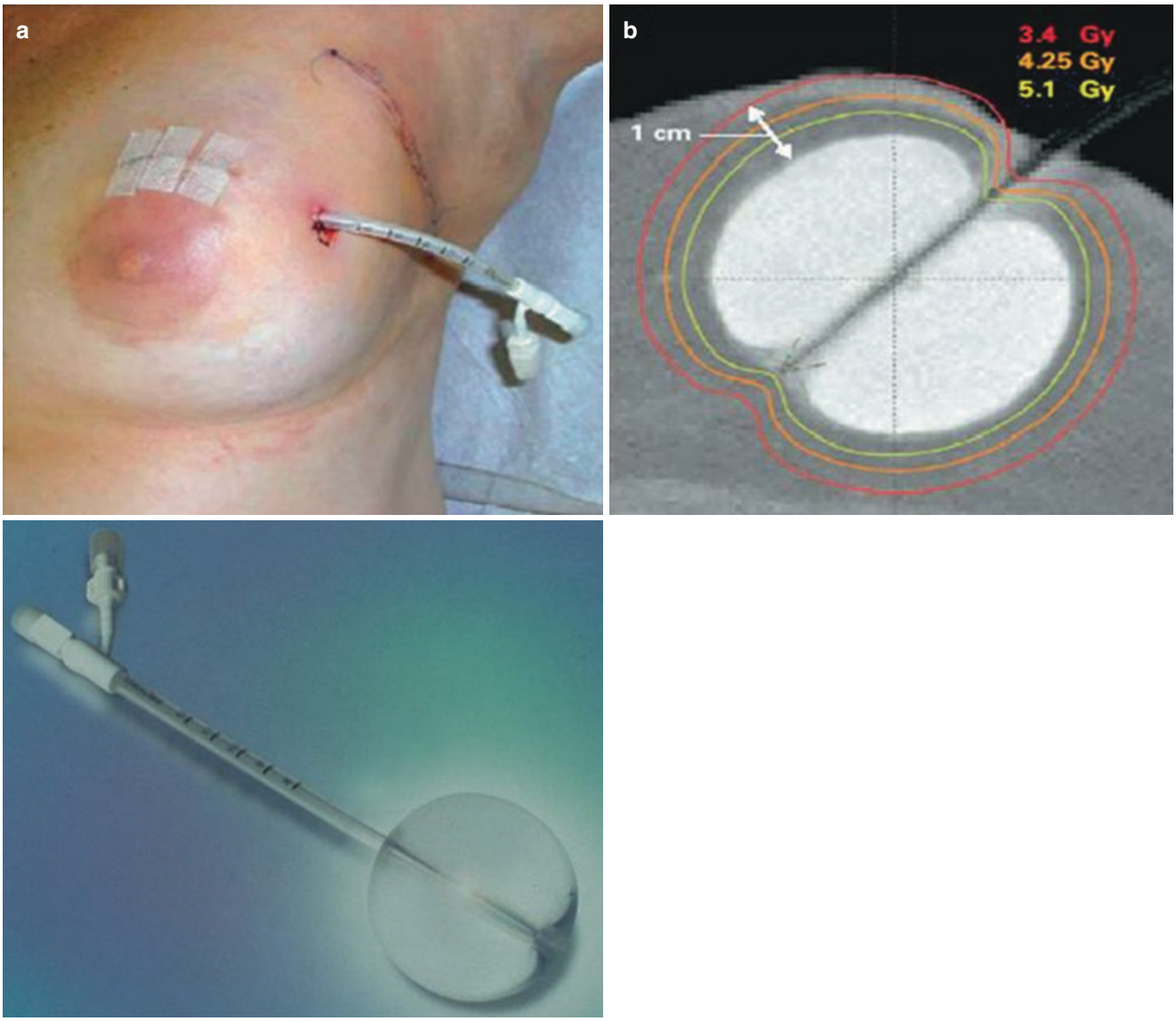

Fig. 55.2 MammoSite system showing balloon placement externally (**a**) and internally (**b**) with dosimetric coverage. The target volume, defined as 1 cm from the balloon surface, is outlined in red. Bottom image (**c**) shows the classic MammoSite applicator [22]

$p = 0.03$). The results of this pilot study lead to FDA approval of MammoSite in May of 2002.

Since that time, over 50,000 women worldwide, including a small proportion of patients with DCIS only, have been treated with MammoSite. The most significant adverse effect that has been seen with greater clinical experience is balloon rupture, with rates that have varied significantly across studies. When this occurs, the balloon is replaced, and the patient is replanned resulting in a short treatment delay. Another potential side effect is persistent seroma, which is more common in patients with higher body weight and adversely affects cosmetic outcome [70]. However, overall results using MammoSite have been excellent, as summarized in Table 55.4. Yet some critiques of these studies, in particular the largest MammoSite registry trial (1449 patients), include (1) possible selection bias due to the voluntary enrolment in the registry study, (2) potential for underreporting of toxicities and tumor recurrence because the data was gathered from multiple institutions, and (3) lack of central pathology review. Nevertheless, the results of these studies in aggregate suggest excellent efficacy and cosmetic outcomes with MammoSite as shown in Table 55.4.

Other single-entry brachytherapy applicators are strut-adjusted volume implant (SAVI) and Contura. SAVI, which was FDA approved in 2006, is a device consisting of a bundle of thin catheters that can be custom fit to the excision

Table 55.4 Results of MammoSite trials

Institution	# of patients	Follow-up (months)	Local recurrence	% Good/ excellent cosmesis
FDA trial [34])	43	66	0% (5 years)	88%
ASBS Registry [35]) [36])	1449	51	2.6% (5 years)	90.4%
University of Wisconsin [37])	26	48.5	3% (5 years)	NR
William Beaumont Hospital [38])	80	22	2.9% (3 years)	88.2%
VCU [39])	483	24	1.2% (2 years)	91%
MUSC [40])	111	46	1% (4 years)	NR
Texas Cancer Center [41])	573	30.5	1% (crude)	96%
Rush [42])	70	26	5.7% (crude)	NR
Kaiser [43])	51	16	0% (crude)	95.6%

FDA Food and Drug Administration, *ASBS* American Society of Breast Surgeons, *VCU* Virginia Commonwealth University, *MUSC* Medical University of South Carolina

cavity. Contura consists of a central lumen and four other lumens, offering a total of 40 dwell positions, encased in a polyurethane balloon. Initial reports on outcomes in patients treated with these devices show excellent local control with acceptable toxicity [44, 45].

55.5.3 External Beam Radiotherapy

External beam radiation therapy is the only noninvasive method of APBI and can be delivered using IMRT or 3D conformal techniques with photons only, combined photons/electrons, or protons. 3D conformal radiotherapy (3DCRT) with photons is currently the most popular technique for delivery of APBI. With the elimination of an additional surgical procedure, 3DCRT is more convenient and with fewer potential complications as compared to invasive techniques. Another potential advantage is improved dose homogeneity within the target volume, which may be associated with superior cosmetic results. Target localization however can occasionally be difficult, especially if the patient develops a large seroma postoperatively or conversely, if a significant amount of time has elapsed between surgery and radiotherapy such that the lumpectomy cavity has been absorbed. This occasionally results in an overestimation (less commonly, underestimation) of the clinical target volume (CTV) to ensure adequate coverage, although clips placed at the time of lumpectomy have been shown to improve the accuracy of cavity delineation [46–48]. An additional PTV margin must be added to account for chest wall movement with respiration, ultimately leading to a larger treated volume than with the other APBI techniques described.

The basic steps for designing and delivering external beam APBI, per NSABP B-39/RTOG 0413 protocol, include the following:

- *Define the clinical target volume and organs at risk on planning CT scan*. A treatment planning CT scan with the patient in the supine position is obtained. The excision cavity is delineated based on clear visualization on CT or with the assistance of surgical clips, if available. Normal structures contoured include the skin, ipsilateral and contralateral breast, thyroid, lungs, and heart.
- *Define CTV and PTV expansions to account for internal motion and daily setup error*. The CTV is a uniform 1.5 cm expansion around the excision cavity, limited to 5 mm from the skin surface and by the posterior breast tissue extent (chest wall and pectoralis muscles are excluded). A 1 cm margin is added to the CTV to create the PTV. The PTV is then copied to a PTV_EVAL which is edited to exclude the portions outside the ipsilateral breast, the first 5 mm of tissue under the skin, and, if applicable, the chest wall, pectoralis muscles, and lung.
- *Determine an appropriate beam arrangement*. Any beam arrangement and number of beams is allowed, as long as necessary dose volume constraints are met. The most common technique is via a 3-, 4-, or 5-field noncoplanar beam arrangement. No bolus to improve anterior target coverage is permitted. For target coverage, 90% of the prescribed dose should cover ≥90% of the PTV_EVAL. The dose limitations for normal tissues are as follows:
  - Uninvolved normal breast: <60% should receive ≥50% of the prescribed dose, and <35% should receive 100% of the prescribed dose.
  - Contralateral breast: no point should receive ≥3% of the prescribed dose.
  - Ipsilateral lung: <15% should receive 30% of the prescribe dose.
  - Contralateral lung: <15% should receive 5% of the prescribe dose.
  - Heart: for right-sided lesions, <5% should receive 5% of the prescribed dose, while for left-sided lesions, the volume of the heart receiving 5% of the prescribed dose should be less than 40%.
  - Thyroid: maximum point dose of 3% of the prescribe dose.
- *Prescribe treatment* to 38.5 Gy, given in twice daily fractions, separated by at least 6 hours.
- *Deliver treatment*. At a minimum, portal films or images of each beam and an orthogonal pair should be obtained prior to initiation of treatment. Orthogonal pair films must also be obtained prior to fraction number 5.

Our practice is similar to the NSABP B-39/RTOG 0413 technique with several notable exceptions. First, our preference is for two non-divergent mini-tangents delivering approximately 80% of the total dose with the rest of the dose provided by an en face electron beam. We have found that this three-field technique reduces dose to nontarget breast tissue (Fig. 55.3). In situations where the seroma is deep and would necessitate high-energy electrons (typically >20 MeV) leading to excessive dose to the lung and heart, we use the more commonly prescribed four-field noncoplanar technique. We also offer APBI over 1 week (4 Gy twice daily fractions to a total dose of 36 Gy) or 2 weeks (4 Gy once daily fractions to a total dose of 40 Gy) allowing patients to choose a schedule that is mostly convenience based. These dose fractionation regimens are based off of data obtained from a Phase I/II dose escalation study at Massachusetts General Hospital (MGH) described in Table 55.5. For treatment delivery, we use both X-ray imaging aligned to surgical clips and surface imaging with Vision RT [57] to account for more subtle changes in breast or arm positioning (Fig. 55.4).

There have been few published randomized series comparing whole breast irradiation to external beam APBI that have

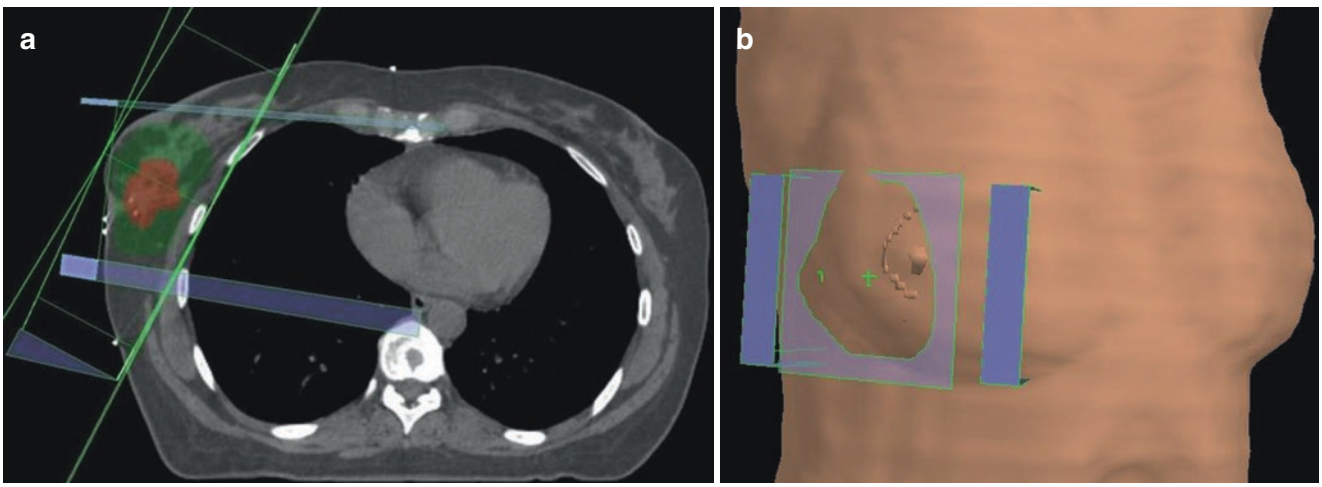

Fig. 55.3 (**a**) Axial CT image showing the use of two tangent fields with an en face electron field. A lateral wedge has been added to improve dose distribution. The seroma is shaded in *orange*, and the target volume is shaded in *green*. (**b**) Surface view of partial breast irradiation field showing en face electron field comprising 20% of the prescription dose. Two mini-tangents are also used delivery 80% of the total dose

Table 55.5 Results of external beam APBI trials

Institution	Dose/# fractions/ frequency	# of patients	Follow-up (months)	Local recurrence	% Good/excellent cosmesis
Photons					
NYU (prone) [49]	30/5/every other day	100	64	1% (4 years)	89%
WBH [50])	38.5/10/BID	94	60	1.1% (4 years)	89%
RTOG 0319 [51]	38.5/10/BID	62	42	6% (4 years)	Not reported
Canadian Multicenter [52]	35/10/BID (n = 9) 36/10/BID (n = 33) 38.5/10/BID (n = 62)	104	36	1% (3 years)	92%
Protons ± photons					
Loma Linda [53]	40/10/daily	100 (protons)	60	0% (5 years)	90%
MGH ([54, 55])	32/8/BID (*n* = 98) 36/9/BID (*n* = 100) 40/10/BID (*n* = 125)	323 (protons *n* = 20, photons *n* = 41, photons/electrons *n* = 262)	52	5% (32 Gy, 4 years) 1% (36 Gy, 4 years) 0% (40 Gy, 4 years)	88% (32 Gy) 81% (36 Gy) 86% (40 Gy)
MGH [56]	32/8/BID	98 (protons *n* = 19, photons/electrons *n* = 79)	82.5	6% (7 years)	62% (protons) 94% (photons/electrons)

NYU New York University, *WBH* William Beaumont Hospital, *BID* twice daily, *RTOG* Radiation Therapy Oncology Group, *MGH* Massachusetts General Hospital

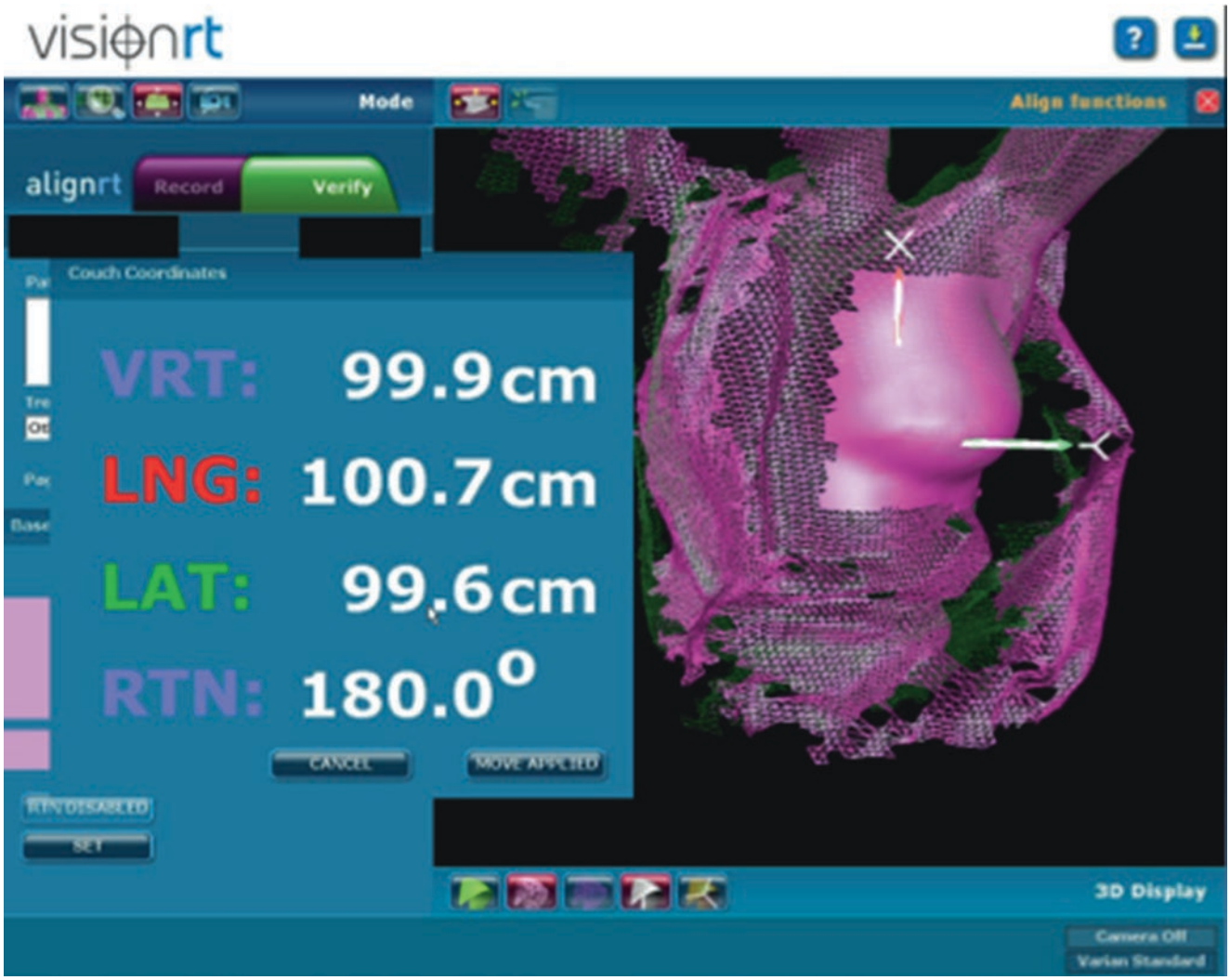

Fig. 55.4 Vision RT. Daily surface images are obtained and compared with reference imaging. Shifts in four directions are calculated and displayed

reported local failure rates. The earliest study was conducted in the United Kingdom in the 1980s and, as described above in the introductory section, showed significantly higher local recurrence rates in the group receiving limited field treatment. Notably, no radiologic imaging was used to define the surgical cavity; thus, the target may have been missed in some patients in the APBI group. Since then, treatment planning has evolved significantly, and more rigorous patient selection criteria have been employed which has led to improved outcomes. Clinical results from select larger Phase I/II studies are summarized in Table 55.5.

As shown in Table 55.5, various dose fractionation schedules for 3D conformal APBI have been used, thus far, the majority of which have resulted in excellent local control. One dose escalation study from Massachusetts General Hospital including 323 patients treated to 32, 36, and 40 Gy in 4 Gy BID fractions showed that local failure rates were low at all three dose levels, but patients treated to 40 Gy had higher rates of fibrosis and fat necrosis [54]. Another dose escalation trial from the Institut Gustave Roussy in France comparing 40 Gy vs. 42 Gy, both in 10 BID fractions, showed both more severe early toxicities and higher rates of late toxicities with the higher dose [58]. At this time, the lowest dose needed to maintain excellent cancer-specific outcomes while optimizing cosmetic results remains unknown. The current national standard, as used in the NSABP B39/RTOG 0413 trial, is 38.5 Gy in 3.85 Gy BID fractions.

Due to the Bragg peak characteristic of proton beams allowing for preferential sparing of normal tissue proximal and distal to the target region, there has been interest in the use of proton radiotherapy for APBI. Dosimetric studies comparing proton versus photon APBI have consistently shown superior proton dose distributions [59–62]. The clinical data are more limited however and stem primarily from a few institutions including Massachusetts General Hospital,

Loma Linda, as well as MD Anderson, as shown in Table 55.5 [53, 54, 56]. Current proton protocols are actively accruing which may demonstrate a clinical advantage to protons [63].

55.5.4 Intraoperative Radiotherapy

Of all the APBI techniques, single-fraction intraoperative radiotherapy performed at the time of lumpectomy is felt by some clinicians to be the most convenient. Some clinicians have also hypothesized that immediate radiotherapy takes advantage of the well-vascularized postsurgical tumor microenvironment, thus maximizing the therapeutic effects of radiotherapy. The two most popular methods of accomplishing this is via photons (TARGIT: targeted intraoperative radiotherapy) or electrons (ELIOT: electron beam intraoperative radiation therapy) as described below.

55.5.5 ELIOT

Patients eligible for the ELIOT trial included women ages 48 and older with tumors 2.5 cm or smaller [64]. After tumor excision, intraoperative radiotherapy was delivered with a mobile LINAC with a 4–8 cm collimator using 6–9 MeV electrons. The total dose to the tumor bed was 21 Gy normalized to the 90% isodose line. The total "beam-on" time was 3–5 min. The chest wall and other normal tissues were protected with a lead/aluminum shield.

55.5.6 TARGIT

With TARGIT, radiotherapy is delivered over 20–45 min via 50 kV X-rays at the center of a spherical applicator that is temporarily sutured inside the surgical cavity [66]. The total dose is approximately 5 Gy at 1 cm (20 Gy at the surface) of the tumor bed. Patients eligible for the trial included women ≥45 years of age with unifocal invasive ductal carcinoma. Those randomized to TARGIT whose pathology revealed adverse features, including margin <1 mm, extensive DCIS, or with invasive lobular histology, were recommended to have supplemental external beam radiotherapy. Of note, to facilitate patient enrolment, the protocol was amended in 2004 to allow for post-lumpectomy randomization with the delivery of TARGIT accomplished via a second open procedure.

55.6 Phase III Clinical Trials

APBI is a promising technique yielding acceptable toxicity and potentially comparable local control to standard whole breast radiotherapy in an appropriately selected patient population. The results from the studies described above have helped to establish preliminary guidelines regarding patient eligibility for APBI. However, to fully understand the applicability of this treatment modality in early-stage breast cancer, large prospective studies are required. At the current time, results from ELIOT, TARGIT, RAPID, and GEC-ESTRO trials have been published as described below:

55.6.1 ELIOT

The ELIOT trial included 1305 women and compared intraoperative radiotherapy to standard whole breast external beam radiotherapy to 50 Gy followed by a 10 Gy boost over 6 weeks. At a median follow-up of 5.8 years, the 5-year recurrence rate for ELIOT met the prespecified threshold for non-inferiority, however was statistically significantly higher than that for whole breast radiotherapy (4.4% vs. 0.4%, $p < 0.0001$). On multivariable analysis, factors associated with local recurrence in the ELIOT group included tumor size >2 cm, presence of ≥4 positive lymph nodes, poorly differentiated tumor, and a triple-negative subtype. The authors concluded that intraoperative electron radiotherapy may be appropriate for women without these high-risk characteristics; however, given significantly increased recurrence rates compared to standard radiotherapy, further prospective validation is needed. Per the updated ASTRO 2017 guidelines, electron beam IORT should be restricted to women with invasive cancer considered "suitable" for APBI [15].

55.6.2 TARGIT

The TARGIT-A study was a randomized controlled trial comparing adjuvant external beam whole breast radiotherapy to risk-adapted intraoperative radiotherapy. The study was powered to assess non-inferiority of the TARGIT regimen of by a margin of 2.5% in local recurrence at 5 years. A total of 3451 patients were randomized and approximately 15% of patients randomized to TARGIT also received whole breast radiotherapy per criteria described above. The local recurrence in the TARGIT group was 3.3% versus 1.3% in the whole breast radiotherapy group ($p = 0.042$), meeting the prespecified threshold for non-inferiority. There were significantly fewer non-breast cancer deaths in the TARGIT group leading to a statistically nonsignificant decrease in overall mortality at 5 years, which the authors argued was due to a reduction in deaths from cardiac causes and non-breast cancers with TARGIT. Based on the results of this study, The National Institute for Health and Care Excellence (NICE) has given preliminary recommendation for the use of TARGIT in the United Kingdom. At the same time, noteworthy criticism has arisen regarding perceived flaws of the study's methodology and interpretation. These include

concerns regarding misuse of the non-inferiority criteria, lack of heterogeneity correction between the groups randomized before and after lumpectomy, and follow-up that is too short to enable conclusions on tumor control and toxicity, particularly in regard to rates of cardiac death and secondary malignancy [65]. Given these concerns, the most recently published ASTRO guidelines on APBI [15] recommend that the use of TARGIT be restricted to patients enrolled in a prospective registry or clinical trial; furthermore, these patients should meet the criteria for the ASTRO "suitable" risk category and have invasive disease only.

In addition, three large multi-institutional trials have completed accrual, and initial results from two of the studies have recently been released. To test the boundaries of patient selection, the inclusion criteria in all three studies are broader than those described in existing guidelines. Of note, none of these studies included intraoperative APBI as a treatment modality.

55.6.3 GEC-ESTRO

The European Brachytherapy Breast Cancer GEC-ESTRO Working Group randomized 1184 women aged 40 years and above to standard whole breast radiotherapy or APBI with HDR or pulse dose rate (PDR) MIB [67]. Core eligibility criteria are listed in the table below. After a median follow-up of 6.6 years, the 5-year local recurrence rates were similar between the two treatment arms (1.44% for APBI vs. 0.92% for whole breast radiotherapy, $p = 0.42$). There was also no significant difference in 5-year regional recurrence, breast cancer-related mortality, or overall survival, which was excellent for both arms at 95.55% for whole breast irradiation and 97.27% for APBI ($p = 0.11$).

55.6.4 RAPID

The Canadian RAPID trial compared whole breast irradiation delivered via a standard or hypofractionated schedule with optional boost to external beam APBI to 38.5 Gy in 3.85 Gy twice daily fractions [68]. The study accrued a total of 2135 women with characteristics described in Table 55.6. Rates of local recurrence have not yet been released; however, an interim analysis on cosmesis and toxicity was conducted after median follow-up of 36 months with results published in 2013. This analysis revealed that adverse cosmetic outcomes were increased in the APBI cohort when assessed by trained nurses (29% vs. 17%, $p < 0.001$), patients (26% vs. 18%, $p = 0.002$), and by physicians (35% vs. 17%, $p < 0.001$). In addition, although grade 3 toxicities were rare in both treatment groups (1.4% vs. 0%), a significantly higher proportion of patients randomized to APBI experienced grade 1 and 2 toxicities. The authors noted that the volume of breast receiving 95% of the prescribed dose was restricted to 35%, but this proportion may be too large in some breasts. We await the publication of mature study results on both efficacy and toxicity; however, these initial results emphasize the importance of establishing appropriate dose and volume parameters for APBI.

55.6.5 NSABP B39/RTOG 0413

The NSABP and RTOG jointly opened a US-based Phase III trial which is the largest randomized study comparing standard whole breast radiotherapy to APBI using 3DCRT, MIB, or MammoSite. The treatment modality will be determined based on physician recommendation, patient preference, and technical feasibility at each study center. This non-inferiority trial estimated a 6.1% 10-year recurrence rate for whole breast irradiation and was powered to detect a 3% higher local recurrence rate with APBI to exclude inferiority. In general, eligibility criteria for the NSABP/RTOG study are less restrictive than the GEC-ESTRO study. For example, there was no restriction on age, and patients with up to three lymph nodes involved were eligible. The original target size was 4300; however, the study was closed after a total of 4214 patients were randomized due to slow accrual. Seventy-one percent of patients were treated with 3DCRT, 23.3% with MammoSite, and 5.7% with MIB (F. Vicini, personal communication). Table 55.6 shows the eligibility criteria and results of the largest modern Phase III trials comparing APBI with whole breast irradiation.

Conclusion

Tremendous progress has been made in the field of APBI since its inception in the 1980s. Given its greater convenience, APBI has become an increasingly popular alternative to standard whole breast irradiation for women who choose breast-conserving therapy. While the earliest studies on APBI showed high local recurrence rates, with advancements in surgery, pathology, and with modern radiation techniques, both the efficacy and toxicity rates have improved significantly. Nevertheless, the majority of long-term published data on APBI are limited to single-arm prospective studies; thus, some clinicians express hesitancy in recommending APBI and the existing guidelines for off protocol eligibility remain stringent. We eagerly anticipate the near future publication of mature 620 results from multiple large randomized controlled trials including over 16,000 women [69] which will further define patient selection criteria, clarify details of treatment techniques including dosimetric parameters, and determine which APBI technique is appropriate for each clinical setting.

Table 55.6 Modern Phase III trials comparing APBI to WBI

Trial name	Location	Accrual dates	Patient inclusion criteria	Surgery	Control arm: WBI	Test arm: APBI	Median follow-up	Patient number	Local failure	Toxicity
National Institute of Oncology	Hungary	1998–2004	>40 years, invasive carcinoma, pT1, cN0, pN0-1mic, grade ≤2, unifocal, no EIC; after year 2001	Wide excision, negative margins	42–50 Gy/21–25 fx	MIB HDR 36.4 Gy/7 fx or electrons 42–50 Gy/21–25 fx	10 years	258	10-year IBTR: APBI 6% vs. 5% WBI ($p = 0.50$)	Excellent-good cosmesis: 81% APBI vs. 63% WBI ($p = 0.009$)
TARGIT-A	Multi-country	2000–2012	≥40 years, invasive carcinoma, T1 and small T2, N0/1, unifocal; no EIC, cN0	Wide local excision	40–56 Gy ± 10–16 Gy boost	50 kV X-rays: 5–7 Gy at 1 cm/1 fx	2 years	2232	4-year IBTR 1% APBI vs. 1% WBI ($p = 0.41$)	Skin breakdown or delayed wound healing: 3% APBI vs. 2% WBI ($p = 0.155$); RTOG grade 3/4 toxicity: 1% APBI vs. 2% WBI ($p = 0.002$); seroma requiring >3 aspirations: 2% APBI vs. 1% WBI ($p = 0.012$)
ELIOT	Italy	2000–2007	>45 years, invasive carcinoma tumor <2.5 cm	Quadrantectomy and ALND or SLNB	50 Gy/25 fx + 10 Gy boost	Intraoperative electrons: 21 Gy/1 fx	5.8 years	1305	5-year same quadrant failure: 3% APBI vs. 1% WBI; 5-year elsewhere failure: 2% APBI vs. 0% WBI	Overall toxicity less with APBI (skin erythema, dry skin, hyperpigmentation, breast edema, breast itching); WBI and APBI equivalent for skin atrophy, fibrosis, retraction, pain, burning; higher rates of fat necrosis and keloids seen with APBI
RAPID	Canada	2006–2011	≥40 years, DCIS or invasive carcinoma if tumor ≤3 cm, pN0	Breast-conserving surgery (BCS) with negative margins, negative ALND or SLNB	42.5Gy/16 fx or 50 Gy/25 fx (if large breast size) ±10 Gy boost	3DCRT 38.5 Gy/10 fx BID	Not reported	2135	Pending	3-year nurse-assessed poor-to-fair cosmesis: 29% APBI vs. 17% WBI ($p < 0.0001$); grade 1/2 late radiation toxicities increased with APBI vs. WBI; grade 3 toxicities rare in both groups (1.4% APBI vs. 0% WBI)

NSABP B39/ RTOG 0413	United States	2004–2013	All ages, DCIS or invasive carcinoma if ≤3 cm, pN0-N1, ECE negative	BCS with negative margins	50–50.4 Gy/25–28 fx ± boost to 60–66.6 Gy	3DCRT 38.5 Gy/10 fx BID, MIB 34 Gy/ 10 fx, MammoSite 34 Gy/10 fx	Pending	4216	Pending	Pending
GEC-ESTRO	Multi-country	2004–2009	≥40 years, invasive carcinoma or DCIS (if Van Nuys prognostic index <8), ≤3 cm, pN0-N1mic	BCS with margins >2 mm for non-lobular invasive, >5 mm for invasive lobular, >5 mm for DCIS	50–50.4 Gy/25–28 fx + 10 Gy boost	MIB HDR 32 Gy/8 fx or 30.3 Gy/7 fx BID or 50 Gy with pulses of 0.6–0.8 Gy/hr. via PDR brachytherapy	6.6 years	1184	5-year local recurrence 1.44% APBI vs. 0.92% WBI ($p = 0.42$)	5-year grade 2–3 late side effects 3.2% APBI vs. 5.7% WBI ($p = 0.08$), 5-year risk of grade 2–3 subcutaneous tissue late side effects was 7.6% APBI vs. 6.3% WBI ($p = 0.53$). 5-year risk of grade 3 fibrosis 0.2% WBI vs. 0% APBI ($p = 0.46$)
IMPORT LOW	United Kingdom	2007–2010	>50 years, invasive carcinoma <2 cm, pN0, grade 1–2	BCS with margins >2 mm	40 Gy/15 fx	3DCRT 36 Gy/15 fx to whole breast with simultaneous 36 Gy/15 fx to tumor bed OR 40 Gy/15 fx to tumor bed only	Pending	2018	Pending	Pending
[a]IRMA	Italy	2013–current	>49 years, invasive carcinoma <3 cm, pN0–1, grade 1–3	BCS with margins >2 mm	50 Gy/25 fx	3DCRT 38.5Gy/10fx	[a]Pending	Target = 3302 (IRMA and SHARE together)	[a]Pending	[a]Pending
[a]SHARE	France	2010–2015	≥50 years menopausal, invasive carcinoma ≤2 cm, pN0-N(I+), grade 1–3	BCS with margins ≥2 mm + clips in place	50 Gy/25 fx + 16 Gy boost, 42.5 Gy/16 fx or 40 Gy/15 fx	3DCRT 38.5Gy/10fx	[a]Pending	1006	[a]Pending	[a]Pending

WBI whole breast irradiation, *APBI* accelerated partial breast irradiation, *MIB* multicatheter interstitial brachytherapy, *HDR* high-dose rate, *EIC* extensive intraductal component, *RTOG* Radiation Therapy Oncology Group, *ALND* axillary lymph node dissection, *SLNB* sentinel lymph node biopsy, *DCIS* ductal carcinoma in situ, *3DCRT* 3D conformation radiation therapy, *BID* twice daily, *ECE* extracapsular extension, *BCS* breast-conserving surgery, *NSABP* National Surgical Adjuvant Breast and Bowel Project, *GEC-ESTRO* The Groupe Européen de Curiethérapie (GEC) and the European Society for Radiotherapy and Oncology (ESTRO)

We acknowledge Dr. Yazid Belkacemi for assistance with this table

[a]IRMA and SHARE have merged for a target accrual of 3302 patients. SHARE contributed 1006 patients and closed in 2015

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Intraoperative Radiotherapy with Electrons (ELIOT)

56

Maria Cristina Leonardi

56.1 Introduction

Intraoperative radiotherapy (IORT) refers to the delivery of irradiation during a surgical procedure, immediately before or after the removal, radically or not, of the tumor mass. The IORT in single fraction can represent the whole treatment, or it can act as an anticipated boost to the most critical areas, followed by completion external radiotherapy.

The advantages of IORT are represented by:

- Direct visualization of the target area, allowing for maximum precision in delivering the treatment
- Sparing the surrounding structures and organs, by displacing or shielding them
- Delivering a single high dose in concomitance with surgery, possibly preventing repopulation from the neoplastic clones in the interval between surgery and subsequent adjuvant irradiation
- Better integration of radiotherapy with systemic treatment
- Shortening the whole radiation treatment, with significant impact on workload of radiotherapy (RT) centers, overall costs, and patient convenience
- Homogeneous dose distribution

These advantages can be applied to the treatment of early-stage breast cancer (BC). In the modern era, IORT is included among the accelerated partial breast irradiation (APBI) modalities, as sole treatment [1].

IORT can be performed by means of megavoltage electrons and kilovoltage photons. This section addresses IORT with intraoperative electrons (ELIOT).

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56.2 Brief History

In 1906, the first intraoperative radiation treatment was performed on a pelvic tumor surgically exposed and submitted to dermopexy, by using low-energy X-rays, by Carl Beck [2]. It consisted actually in external roentgen treatment of internal structures and was carried out several times. The need of bringing tissues directly in contact with the radiation source came from the fact that deep-seated tumors challenged the delivery of high dosage while keeping the morbidity of treatment low, at that time when only low-energy photons were available. It was only several years later that the concept of IORT as a single fraction immediately after tumor removal emerged, but the technical difficulties of the procedures still restricted its widespread use [3]. The intraoperative techniques continued to be refined over the subsequent years, with the introduction of dedicated applicators and equipment. At the end of the 1940s, Fairchild and Shorter combined IORT with adjuvant external beam radiotherapy for inoperable gastric cancer [4], demonstrating the feasibility of integrating the two modalities. In the 1960s, Abe and collaborators at Kyoto University Hospital in Japan [5] started using IORT with electrons as the sole treatment, using doses of 20–40 Gy, which reduced the exit dose to normal tissues, introducing the advantage of megavoltage intraoperative irradiation over orthovoltage irradiation. Later on, in the 1970s in the USA, the role of IORT with electrons as a boost was investigated, followed by external beam doses. In the early 1980s, interest in IORT was also shown in Europe [6].

However, some logistical difficulties still limited the application of IORT. Patients with an open surgical wound and under anesthesia had to be moved from the surgical theater to the radiotherapy department, where the linear accelerator was located, with concerns related to sterility, anesthetic surveillance, and lengthening of the surgical time.

In the mid-1990s, the development of miniaturized mobile accelerators, which are placed directly in the operating theater with no need of special structural modifications for radioprotection, dramatically facilitated the procedure and

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opened the way to a more extensive use of IORT. These dedicated linear accelerators of reduced weight and size produce electron beams with energies nominally comprised in a variable range from 3 to 12 MeV and are characterized by high-dose rates, which reduces the duration of the treatment to a few minutes.

The electron beam is collimated by means of applicators of cylindrical geometry, made of perspex or plastic material, whose sterile terminal part, during treatment, is placed in contact with the tissues to be irradiated. The components are made of materials which minimize the production of bremsstrahlung X-ray and scattered radiation in the surrounding environment, thus limiting the precautions linked to radioprotection.

The main fields of application of IORT consist of rectal, gastric, gynecologic cancers, and sarcomas. Reports on the use of IORT in breast cancer are less numerous. First experiences in Europe and the USA date back to the early 1980s, focusing on the use of IORT as a boost in combination with breast-conserving surgery. The feasibility of IORT was assessed on the basis of postoperative recovery, tolerance of the subsequent postoperative RT, cosmetic results, and local control. Highly positive results were reported in the first series of patients during the 1990s, showing a great potential for a larger application of IORT in breast cancer. In 1998 the International Society of Intraoperative Radiation Therapy (ISIORT) was set up to promote basic and clinical research programs and develop cooperative studies involving the use of IORT. Currently numerous centers worldwide are affiliated to the ISIORT [7]. In 2006 the European section of ISIORT (ISIORT-Europe) was established.

56.3 ELIOT in Breast Cancer

56.3.1 Intraoperative Procedures

After the excision of the breast tumor, the surgeon mobilizes part of the remaining breast around the tumor bed by separating the deep side from the fascia of the pectoralis major muscle and the superficial side from the subcutaneous tissue at the level of the anterior adipose lamina. To protect the chest wall and the deep-seated thoracic organs, a dedicated aluminum-lead shielding disk, available in various diameters, is placed between the gland and the pectoral muscle (Fig. 56.1). The breast anatomy is temporarily restored by bringing together the section areas of the excision with a line of sutures, in order to expose the most homogeneous surface to the radiation beam. The electron beam energy is selected in accordance with the thickness of the target tissue, which is measured with a needle and a ruler. The irradiation is delivered through Perspex cylindrical applicator with different diameters, from 3 to 12 cm, either flat or beveled (Fig. 56.2).

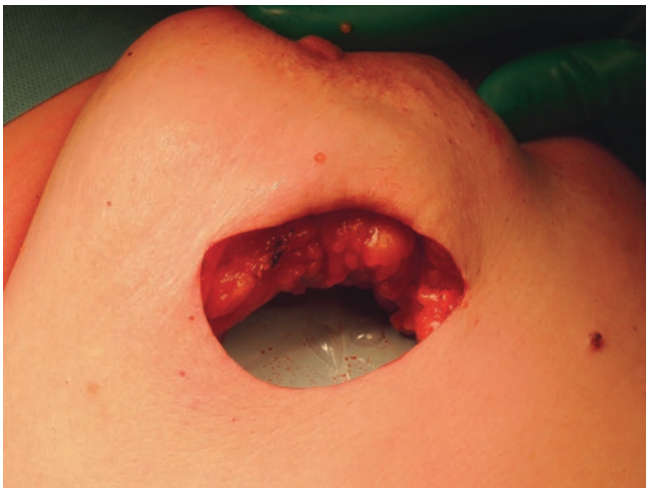

Fig. 56.1 After tumor removal, an aluminum-lead disk is placed over the pectoralis major fascia and under the gland, to shield the underlying chest wall structures

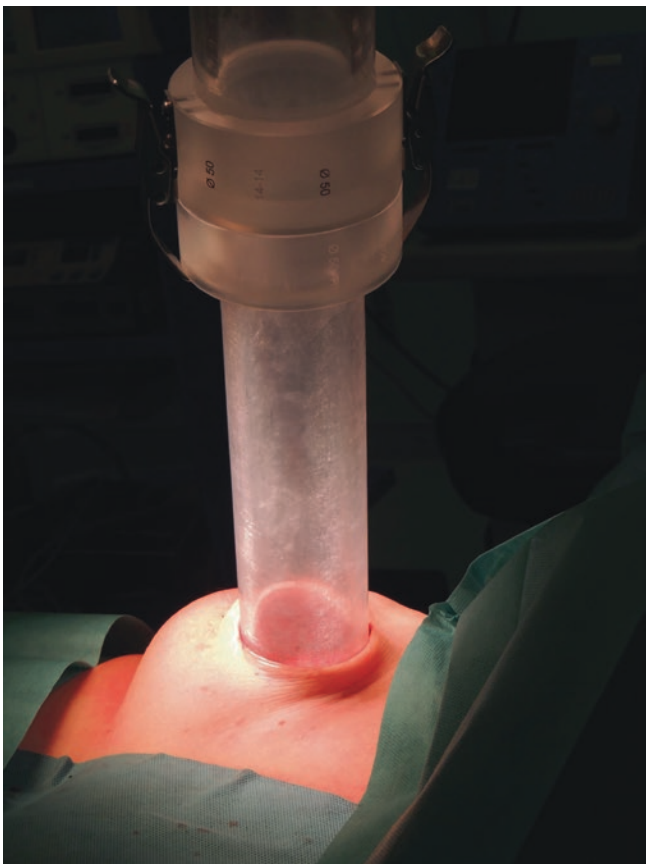

Fig. 56.2 The applicator is inserted through the surgical breech directly in contact with the breast tissue

The applicator is chosen in order to guarantee the proper coverage of the entire target volume, which is an area of 4–6 cm of diameter around the surgical sutured breech, depending on the tumor size and location. The applicator,

Fig. 56.3 The mobile linear accelerator in the operating theater, ready to deliver intraoperative radiotherapy

attached to the gantry, is placed directly in contact with the breast gland ("docked"), moving the linac by remote control (Fig. 56.3). If the operating theater walls are not structurally shielded, mobile barriers are positioned around (2-cm-thick lead shields, 100 cm long and 150 cm high) and beneath the operating table (primary beam stopper, a trolley-mounted 15-cm-thick lead shield) to provide a good shielding of X-rays scattered by the patient, the components of the linear accelerator, and the table itself. The whole irradiation lasts about 2 min. After delivery of the dose, the applicator is removed along with the shielding disk by undoing the temporary sutures, and the incision is closed again in the conventional fashion [8–10]. Some tumor locations are not suitable to be treated with ELIOT due to insufficient residual

breast parenchyma or the marginal localization, such as the axillary tail, the inframammary fold, or close proximity to the skin.

Among all the perioperative radiation techniques, namely, multicatheter brachytherapy, endocavitary brachytherapy, orthovoltage system, and ELIOT, the latter one ensures the best homogeneity of dose distribution within the planning target volume. In addition, the average dose delivered to the target volume by ELIOT was the closest to the prescribed dose, while the average dose outside the target was the smallest, obtaining the best dose sparing of the surrounding tissues. However, ELIOT, using a round central applicator, presents a strictly centric dose falloff, which makes it less adaptable to irregular-shaped target volume. This symmetric spatial dose delivery does not take into consideration the irregular spread of microscopic disease and the variability in surgical margin distance from the gross tumor, which only becomes available on the final histologic report [11].

ELIOT demands good multidisciplinary collaboration and quality assurance protocols, which define the organizational and operational aspects of the procedure [12, 13]. Continuous training for the personnel involved with a clear definition of roles and responsibilities, strict program of quality control for the equipment concerning all the physical and dosimetric characteristics of the electron beam, and guidelines to define patient selection, and prescription dose criteria must be established in each RT center [14].

In vivo dosimetry, which is routinely used in external RT, can be performed also for ELIOT, taking into account the particular conditions occurring in the operating room (need of sterility, field perturbation, etc.). By using radiochromic films to measure the entrance dose, although not giving an immediate redout of the dose, the agreement between measured and expected dose can be checked. Also a real-time procedure with micro-MOSFET detectors seems to be feasible and reliable, although with some technical limitations [15, 16].

56.3.2 Dosimetric Considerations

Biological equivalent dose (BED) is a measure for comparing the expected biological effect of different fractionations. The BED is given by

$$BED = \frac{nd\left[1+d\right]}{\alpha / \beta}$$

where n and d are the number of fractions and dose per fraction, respectively, and α/β ratio is the fractionation sensitivity.

When a tissue receives a higher dose than that prescribed, an increase in BED occurs with potential worsening of normal tissue toxicity. This effect is known as "double trouble" when conventional fractionation of 2 Gy is used, but it is even more problematic ("triple trouble") if larger dose/fraction is given, because a greater increase in BED results [17, 18].

The BED values of most APBI protocols resulted in tumor control BEDs lower than 60-Gy conventional schedule, being around 50 Gy given in 2 Gy/fraction. The critical organs' BED values were lower than the standard schedule with regard acute toxicity and roughly equivalent for late toxicity [19]. Conversely, when IORT was considered in comparison with the 60-Gy conventional fractionation, both the tumor and the normal tissue BEDs were higher. In particular, the normal tissue BED was as high as the double compared to 60 Gy standard fractionation (241 Gy vs. 120 Gy, using α/β ratio of 2 Gy). In theory, an excess in normal tissue damage by 21 Gy IORT would be expected due to a saturation of the repair mechanisms, but so far there is no evidence of severe side effects in the clinic.

The mathematical model used to derive the BED is based on the linear-quadratic (LQ) formalism: i.e., the effect of any single dose of RT depends on a weighted sum of the dose (with weighting coefficient α) and of the dose squared dose (with weighting coefficient β).

The LQ equation is the most used and the simplest model for calculating isoeffect doses for different fractionations. There are some concerns regarding whether LQ model can describe dose responses in the dose range of hypofractionated schedules. Based on experimental and empirical observations, LQ model is predictive of dose-response relations in the dose per fraction range of 2–10 Gy. Above 10 Gy, the model seems to become less accurate, but still acceptable for dose per fraction of 15–18 Gy. For ELIOT full dose of 21 Gy, LQ model should be not adequate for predicting fractionation effects. Moreover, the value of α/β ratios is not given once and for all, but must be fitted on clinical data and differ for different endpoints. Even for a single endpoint, wide confidence intervals of the α/β ratio are reported in literature.

In addition, most APBI schedules influence the biological mechanisms of cell response to radiation, represented by the 5Rs (repair, redistribution, reoxygenation, repopulation, radiosensitivity) [20, 21]. Although the tumor BED for IORT 21 Gy is higher than that of standard schedule (131 Gy vs. 90 Gy, using α/β ratio of 4 Gy), the single fraction delivered at a high-dose rate does not allow the reoxygenation effect and the redistribution of cells through the cell cycle. As a result, the less radiosensitivity might affect the local control [22]. On the other hand, the delivery of a high dose immediately after surgery might interfere with the tumor microenvironment and prevent repopulation from the neoplastic clones

in the interval between surgery and subsequent adjuvant irradiation [23].

Regarding ELIOT as the sole treatment, virtually all the clinical studies use 21 Gy as standard dose. The most common point of prescription is at the isodose of 90%, while some authors prefer at the depth of maximum dose (Dmax). The biological equivalent dose is felt to be 1.5–2.5 times higher than the dose delivered with external beam RT [24]. By applying the LQ model [25], using α/β ratio of 4 Gy for breast tumor, the single dose of 21 Gy appears to be equivalent to 65 Gy given with 2-Gy fractionation.

Regarding ELIOT as a boost, a wide variety of doses are described in the literature, mostly ranging from 6 Gy to 15 Gy. By applying the linear quadratic model, using α/β ratio of 4 Gy for breast tumor, the equivalence to the 2-Gy schedule for such a dose range falls in between 11 and 37.5 Gy.

From 2007 the ISIORT-Europe centers were asked to fill in an IORT registry available at the ISIORT website with clinical and technical data of patients affected by any type of cancer treated with IORT either with electrons or low-energy photons. Regarding breast cancer, data from 2395 patients were collected, providing an insight on the European clinical practice. Only one third of the patients entered clinical trials for the single dose, and even fewer in case of boost. IORT dose used as single treatment was in the range of 18 Gy (8%), 20 Gy (23.8%), and 21 Gy (71.1%), while IORT boost ranged between 8 and 12 Gy [26].

56.4 ELIOT as the Sole Treatment

The rationale of APBI is based on the fact that the majority of residual neoplastic cells are in the vicinity of the primary tumor, after breast-conserving surgery (BCS). Data from mastectomy studies showed a relationship between the index tumor and the occult multifocal and multicentric malignant disease. In the study performed by Holland [27] on tumors 4 cm or less in diameter, in 90% of cases, neoplastic foci were restricted within 3 cm from the edge of the index tumor. This pathological finding is supported by the clinical evidence that the majority of local relapses, after conservative treatment, arise in close proximity to the primary tumor [28, 29]. Therefore, in selected patients, limiting the radiation target volume to the area at higher risk should achieve local control equivalent to whole-breast radiotherapy (WBRT). Over the past 10–15 years, the partial breast radiation has been gaining ground in the treatment of early breast cancer. Among the various APBI modalities, ELIOT is an attractive approach and offers a number of advantages, but the real challenge now is to be able to select patients who would benefit most from this intraoperative modality.

The possibility of replacing the entire treatment of 5–7 weeks with a single session carries a positive impact on medical and social costs of treatments. Cost analysis studies demonstrated the cost-effectiveness of IORT as a single procedure over a long course of WBRT [30]. Even taking into account the capital investment for the equipment, IORT itself is a cost-saving procedure and provides greater quality of life. However, a warning comes from the higher risk of local relapse compared to whole-breast radiotherapy, which eventually leads to increased overall costs, jeopardizing its cost-effectiveness. A careful patient selection is advocated to maintain the economic advantage.

56.4.1 An Overview Across the Literature

In virtually all the clinical trials, ELIOT full dose was given in peri- or postmenopausal patients. Across the literature, the minimum age for APBI was 45 years, but most studies included patients aged 48 and over, while only one study considered eligible patients from the age of 40. At the study conducted at the University of Verona, Italy [31, 32], patients over 60 were enrolled, whereas Lemanski investigated the feasibility of IORT full dose in patients aged ≥65 in a small phase II trial conducted in Montpellier, France (the RADELEC trial) [33, 34]. The RADELEC trial was dedicated to very low-risk tumor profile: T1N0M0, unifocal, ductal invasive, positive estrogen status, and age ≥65 years old. Forty-five patients entered the study. The median age was 72 years. Four patients had local events (three in the same quadrant as the index tumor), with a median follow-up of 72 months, and underwent salvage standard mastectomy. Among patients treated off-protocol at the European Institute of Oncology (IEO), Milan, Italy [35], age under 50 emerged as a significant factor for local recurrence. Due to the limited flexibility of ELIOT field correlated with the surgical breech extension and the available collimators, ideally the maximum tumor size should be 2 cm. Tumor greater than 2 cm was found correlated with local relapse in the ELIOT randomized phase III trial carried out at IEO, Milan [36], but this study paid the price of having used too small collimator size (4 cm as median diameter). In the study from the University of Verona [31], tumors larger than 2 cm were included, but a median collimator size of 6 cm was used, and care was taken to ensure that the diameter was roughly 2 cm greater than the largest tumor dimension. The eligibility criteria included ≥50-year-old patients, with tumor size of ≤3 cm, any grade, any estrogen receptor status, unifocal ductal carcinoma, and radically excised. At a median follow-up of 62 months, 4/226 patients developed local recurrences. Mussari and colleagues from Trento, Italy [37], reported on 47 patients treated with three different dose levels: 20 Gy (at 90% and 100% isodose), 22 Gy (at 100% isodose), and 24 Gy (at 100% isodose). The eligibility criteria included age >45 years, T1N0 up to 2 cm,

clinically negative axillary nodes, G1-G2, positive hormonal status, and no intraductal component at preliminary biopsy. After median follow-up of 48 months, no local relapse was found. The collimator diameter was 5–6 cm. The most common applicator used in the study from Brussels, Belgium [38], was of 5 mm in diameter. The investigators tried to adapt the intraoperative planning target volume to the tumor size, by applying the rule of increasing the field diameter by 4 cm compared to the tumor diameter. Therefore, for pT1a tumor, the field size was at least 36 mm, while for pT1c tumor, the field size was 46 mm. One local recurrence occurred in a quadrant of the breast not originally involved. The actuarial rates for disease-free survival and overall survival and disease-specific survival were 97.6%, 98.9%, and 98.9%, respectively. Five-millimeter diameter was also used by Osti and colleagues from Rome, Italy [39], who evaluated the effectiveness and the tolerance of 21-Gy full dose (prescribed at 90% or 100% isodose curves) in 110 patients, with the same inclusion criteria as ELIOT trial (tumor size <2–5 cm, age >48 years, postmenopausal status). Three local recurrences (2.7%) occurred with actuarial local control rate at 2 and 3 years of 98.4% and 94.5%, respectively. Out of three local relapses, two were true recurrence (1.8%) and one was suggestive of new ipsilateral tumor.

Beyond the width of the intraoperative radiation field, another critical issue regards arranging the cylindrical applicator so that the central axis coincides with the center of the original tumor site. Not always surgical excision has the lump perfectly at the center of the breech, and, once the breast is temporary reconstructed, the collimator cannot ensure equidistant margin from the former tumor site [40].

To address the problem of the correct identification of the tumor bed once the tumor has been excised, the group from University of North Carolina adopted the approach of delivering ELIOT before tumor removal [41, 42]. In this series of 53 patients, aged ≥48, with invasive ductal carcinoma, ≤3 cm, nodal negative, a single dose of 15 Gy was given to the intact tumor, before excision. Median FU was 69 months. A total of eight local recurrences (five of them were true recurrences) for a crude rate of 15.1% were observed. By applying the ASTRO Accelerated Partial Breast Irradiation Consensus Statement Criteria [43], the local event rates were 5% in the suitable, 27% in the cautionary, and 0% in the unsuitable group, without statistically significant difference. The overall survival was 94.45 and the breast cancer-specific survival was 100%.

Few studies did not consider poorly differentiated tumors (grade 3) as eligible for ELIOT [37]. In the ELIOT phase III randomized trial [36], grade 3 tumors were predictors for local recurrence. In the University of Verona study [31], which included any grade, two out of the four locally relapsed tumors were of grade 3.

Estrogen receptor status was predictor of local failure in the ELIOT phase III trial [36], but it was not a uniform criterion of exclusion in all the studies.

Histology other than ductal carcinoma was excluded in some studies [31, 34, 44] and included in others, without detecting significant differences [37–39]. In the ELIOT phase III study [36], the extensive intraductal component (EIC) did not result in increased risk of local relapse. Other trials [31, 34, 39] did not consider EIC among the eligibility criteria for ELIOT.

The eligibility criteria of majority of the studies included clinically negative axillary nodes. The study carried out by Cedolini and colleagues from Udine [44], Italy, on 77 patients included among the inclusion criteria N0 or N1mi along with ductal histology, size <3 cm, free margin of >5 mm, and age ≥48 years. At 6 years of follow-up, 2% of local recurrences were described, distant from the index tumor site. However, although clinically negative, some patients turned out to be nodal positive on the final histologic analysis. In the ELIOT randomized phase III trial [36], more than three positive nodes was correlated to an increased risk of local failure. This correlation seemed to disappear in case of limited nodal involvement. In fact, the patient group from the University of Verona study [31], patients having 1–2 positive nodes (22.1%), and the patient group from Udine [44] with minimal nodal involvement (4.1% N1mi and 1.4% pN0i+) did not present any increase in risk of local failure.

56.4.2 The IEO Experience

The IEO extensively developed and implemented this modality of APBI, going through a number of phases aimed at clinical validation of the procedure.

Phase I/II dose escalation. The study began in July 1999 and was closed in April 2000. The primary endpoint was the assessment of tolerance of progressively increasing doses of ELIOT. The first ten patients received an intraoperative boost dose of 10 Gy followed by external beam RT to the whole breast up to 44 Gy in 22 fractions. In seven patients, the boost dose was increased to 15 Gy, while the whole breast received 40 Gy in 20 fraction with external RT. The remaining patients were treated with ELIOT alone, with three different dose levels, 17 Gy (8 patients), 19 Gy (6 patients), and 21 Gy (24 patients), prescribed at Dmax in all the cases. No major acute and intermediate toxicity was observed during a relatively short follow-up. One patient treated with 21 Gy developed an infection, while one patient treated with 10 Gy boost suffered from severe fibrosis. One local relapse was observed after 17-Gy full dose, and one patient in the boost group had bone metastases.

Phase II study. From May to November 2000, 50 additional patients were treated with the single dose which was

prescribed at the 90% isodose. This change in dose prescription increased the Dmax dose from 21 to 23.2 Gy, because it was observed that a small percentage of patients with large breasts had a slight underdosage of the deep part of the target tissue. The aim was to assess the acute and intermediate toxicity. Acute toxicity was low: 15 cases had signs of moderate mammary fibrosis and 4 cases had liponecrosis, in line with the relevant literature. Five cases developed local relapse, two of them in the same quadrant as the primary tumor [29].

Phase III study. The results of the phase I–II studies laid the foundation for the prospective, randomized phase III study (ELIOT trial) designed to assess the equivalence in efficacy between the postoperative conventional radiotherapy and ELIOT full dose (21 Gy).

The randomized phase III trial started in 2000 after being designed in 1999 [36]. The eligibility criteria were very simple and were based mainly on clinical, radiological criteria and on cytology. The trial randomized 1305 patients aged over 48 with tumors smaller than 2.5 cm between WBRT (50 Gy + 10 Gy boost) and a single fraction of 21 Gy directed to the tumor bed using intraoperative electrons. Available for the analysis were 601 and 585 patients in each arm. The two arms were well balanced with each other; only an excess of grade 1 tumors was seen in the IORT arm.

The primary endpoint was local recurrences, which was defined as a sum of local recurrences at the lumpectomy site ("true") and second ipsilateral tumors ("elsewhere") occurring in any breast quadrant. This was an equivalence trial, and the prespecified equivalence margin was a 7.5% rate of local recurrence in the ELIOT arm, assuming a 3% rate of local recurrence in the WBRT arm. After medium follow-up of 5.8 years, ELIOT patients had a higher 5-year recurrence rate than WBRT patients (4.4% vs. 0.4%, $p < 0.0001$). As 60% of local relapses in the ELIOT trial occurred near the primary tumor site, this observation called for a comment on radiation field size. Median applicator size of 4 cm may have been too small to adequately cover larger tumors. Tissues at the periphery of these smaller applicators may have been inadequately irradiated.

In addition, regional lymph node relapse was significantly greater in the ELIOT group, probably due to the lack of tangent field contribution. In contrast, there was no significant difference in the 5-year rates of contralateral breast cancer, distant metastases, breast cancer-specific mortality, and overall survival between the two groups.

For patients in the intraoperative radiotherapy group, the characteristics associated with local relapse were analyzed, to allow identification of patients who might benefit from subsequent whole-breast irradiation.

In multivariable analysis, tumor size greater than 2 cm, the presence of four or more positive lymph nodes, a poorly differentiated tumor, and triple-negative subtype roughly doubled the risk of local recurrence. In fact, the presence of one or more of these risk factors brought up the 5-year local recurrence rate to 11.3%. It is important to note that the ELIOT protocol did not account for adverse final pathology findings, so that no additional treatment was delivered in case of disappointing final histologic report.

All the patients who wished to undergo IORT but did not fulfill the eligibility criteria to enter the phase III randomized trial were treated apart and formed the so-called out-trial population that provided very interesting results. A total of 1822 patients treated off-protocol was analyzed [35]. In this group, the rate of nodal positivity was relevant. At median follow-up of 4 years, cumulative incidence of local recurrence was 3.6%, and dividing it into true and elsewhere recurrence, the rate was 2.3% near or at the original tumor bed and 1.3% elsewhere, very similar to the rate of contralateral breast cancer. The 5-year and 10-year survival was 97% and 90%, respectively. At the multivariate analysis, predictors of local events were young age, namely <50, tumor size of >2 cm, and unfavorable subtypes. To help physicians to select the proper patients for APBI, the American and European radiation oncologists provided some guidelines which stratify patients into three subgroups defined as suitable, to be treated with caution or unsuitable for APBI [43, 45]. These guidelines are based on clinical and histopathologic factors known to be predictive for local recurrence, although some other important features, as Ki-67 or HER2, were not included. Although it is challenging to apply the ASTRO guidelines in the case of ELIOT, because the comprehensive pathologic view is not yet available while delivering intraoperative irradiation, any efforts must be made in collecting as much information as possible in the preoperative setting. It is of utmost importance that core needle biopsy and intraoperative frozen section assessment can detect the most pertinent pathologic information either before or at the time of surgery.

Categorizing out-trial patients into the ASTRO and ESTRO groups [46, 47], ASTRO recommendations well defined the risk groups with statistical differences in the rate of local failure among the three groups. The 5-year rate of ipsilateral breast recurrence for suitable, cautionary, and unsuitable groups was 1.5%, 4.4%, and 8.8%, respectively ($p = 0.0003$). Conversely, ESTRO recommendations, that are less strict than ASTRO, failed to do so. ESTRO criteria identified the good candidates, who experienced 1.9% rate of local relapse, but did not differentiate the intermediate ("possible candidates") and the high-risk ("contraindications") patients, who experienced 7.4% and 7.7% rates of local relapse, respectively. Breaking down the cumulative rate of local failure, in the low-risk group, the rate of true and elsewhere failure was very low (1.6% and 0.6%, respectively), and this category can be safely treated with ELIOT, while the intermediate- and high-risk groups showed a high incidence of both true and elsewhere recurrences (4% and 3.3% and

4.7% and 3%, respectively) that probably only WBRT can optimally handle.

A post hoc analysis conducted among in-trial patients [36] confirmed the effectiveness of ELIOT in the suitable category according to ASTRO criteria 2010. Less than 25% of the analyzed patients fell into ASTRO's suitable category for APBI [43]. In fact, categorizing the patients enrolled in the randomized trial into the three ASTRO groups, the suitable ones fared well, irrespective of the radiation modality. In the study from the University of Verona [32], three out of four failures reported did not meet ASTRO suitable guidelines for APBI. It proves that a proper selection can bring positive results. The strict selection can bring to a drastic reduction in the number of patients fully suitable for ELIOT. The group from Genoa, Italy, applied two-step decision-making procedure: the first decisional step was made after diagnosis and staging and the second one during the surgical procedure on the basis of intraoperative tumor information. In their experience, the ultimate rate of patients deemed good candidate for IORT was 43% [48].

56.4.3 Cosmesis and Side Effects

So far, reports across the literature have shown that ELIOT presents low acute toxicity and acceptable late toxicity. Cosmesis has been scored as good to excellent in the majority of patients. In fact, all the studies describing aesthetic outcome, cosmesis was judged good or excellent in 92–95.5% of the cases [37–39, 49]. The most common side effect reported was breast fibrosis. The severity of fibrosis was described using different scales. Among 1822 out-trial patients, breast fibrosis was scored by using four-point scale (none, light, moderate, and severe). Breast fibrosis was detected in 34 (1.9%) and was scored as severe in 2 of them, while moderate skin retraction was observed in 14 (0.6%), after median follow-up of 36 months [35]. In a small group of 119 patients treated at IEO off-protocol, with a longer follow-up of 71 months, fibrosis was scored according to LENT-SOMA scale [49]. Fibrosis was grade 2 in 32% and grade 3 in 6%. Light persistent or mild intermittent pain was reported in 3.3% of the cases. By applying the LENT-SOMA scale, Philippson and colleagues from Brussels observed late toxicity in 17.1% of the patients who were given ELIOT 21 Gy (90% isodose). Fibrosis was grade 1 in 8.3% and grade 2 in 3.4%. Grade 1 atrophy was described in 5.4% [38].

In the French study by Lemanski [34], side effects were scored using the CTV v.3.0 scale. The study population included elderly patients, who are known to be susceptible to experience worse cosmetic results due to the increased amount of fatty tissue replacing glandular parenchyma [50]. Fat necrosis was frequent: it was observed in 71% of patients, corresponding to a palpable fibrosis in the ELIOT area in 40% of the cases. Ten patients suffered from late grade 1 breast pain and one experienced a rib fracture. Other studies reported lower incidence of radiological liponecrosis [37, 51].

Using the RTOG/EORTC scale for late toxicity, in the study from Trento [37], fibrosis was reported of grade 2 in 30% and of grade 3 in 2% of the cases. A minority of patients complained breast pain (2.1%). In the study from Rome [39], fibrosis was defined as mild in 4.5% of patients, and 5.5% had moderate skin retraction. The authors noticed that over time there was a tendency of gradual attenuation in the severity of fibrosis.

The most common postoperative complications reported in the studies included edema (1.5%), hematoma (1.5–12%), seroma (8%), light to moderate pain (1.3–14%), wound complications like dehiscence, delayed cicatrization (1–7%), and infection (0.4–2.3%) [31, 36, 38, 49]. Clinical liponecrosis, which was a localized collection of brown fluid with skin erythema, was observed in 2–15.5% [36, 37, 39].

In the study from the University of Verona, at 6 months after IORT, 31.4% presented breast asymmetry involving ≤1/3 of the gland, while in 8.4% of the patients asymmetry was greater than one third of the breast volume.

Pulmonary fibrosis was uncommon among IORT patients, because of the aluminum-lead shielding disk beneath the reconstructed gland [52].

56.5 ELIOT as a Boost

56.5.1 An Overview Across the Literature

The role of the boost in reducing the incidence of local recurrence has been widely confirmed by the final data of the EORTC trial, which showed a significant increase in local control in the group receiving 16 Gy boost compared to that receiving WBRT alone [53]. This benefit was particularly significant for younger patients. In the era of oncoplastic surgery, where the mammary gland is reshaped after conservative surgery to obtain the best cosmetic outcome, the delivery of the boost dose with an intraoperative technique is quite relevant, as the original tumor bed can no longer be radiologically or anatomically evident. ELIOT boost prevents missing the target or enlarging the boost area, which might lead to more extensive fibrosis and impaired aesthetic result. Moreover, the partial sparing of skin and subcutaneous tissues could limit dyschromias and telangiectasia. In addition, the intraoperative boost reduces the overall treatment time with external whole-breast irradiation by 1–2 weeks. Feasibility studies carried out in Montpellier, France [54], and at the Medical College of Ohio, Toledo, USA [55, 56], on small groups of patients (51 and 21, respectively) affected by stage I or II breast cancer were published in the late 1990s

and were satisfactory in terms of efficacy and toxicity. In the French study by Dubois and colleagues, 51 patients were treated with IORT at a dose of 10 Gy and, after an interval of 10–15 days, with external RT on the entire breast to 45 Gy. With a minimum follow-up of 2 years, there was no local recurrence and the aesthetic result was acceptable, reporting only three cases of subcutaneous sclerosis with negative aesthetic impact. The study was updated in 2006 by Lemanski [57], who reported, after a median follow-up of 9.1 years (range 5–15 years), two local recurrences within the primary tumor bed. Six patients complained grade 2 subcutaneous fibrosis in the boost area, and other two patients experienced grade 1 telangiectasia. No grade 3 side effects were detected, and cosmesis was good to excellent in all the patients. The clinical experience in the USA, performed at the Medical College of Ohio, Toledo [55, 56], regarded 21 patients who were treated in the period 1984–1996 with 10 Gy (18 patients) and 15 Gy (three patients) intraoperative boost and subsequently with 45–50 Gy with external beam radiotherapy to the entire breast. Cosmesis was excellent (only two cases of palpable fibrosis), and after a median follow-up of 71 months, there was no local recurrence. These studies favored the boost dose of 10 Gy as the most tolerable in combination with external WBRT. The boost dose 10 Gy was adopted by other American institutions. St Joseph Hospital, in California [58], reported low acute toxicity on 50 patients with median follow-up of 10 months, treated with subsequent WBRT with different dose (40–50.4 Gy). A similar number of patients were irradiated at the Mayo Clinic in Arizona [59] with the same approach (10 Gy ELIOT boost and 48 Gy external WBRT). At a median follow-up of 79 months, two patients had local recurrences. At univariate analysis, HER2 overexpression and the presence of extensive intraductal component were significantly associated with local failure. Most toxicities improved with time. At last follow-up cosmesis was judged good to excellent in 86% of cases and poor in two patients (4%). One patient developed severe fibrosis and breast deformation after aspiration of a symptomatic seroma. The 6-year actuarial overall survival and distant control rate were 89% and 96%, respectively.

In the Austrian retrospective comparative study, the boost dose delivered with ELIOT seems to be even more effective than that given immediately after the completion of WBRT [60, 61]. Three hundred and seventy-eight women affected by stage I and II breast cancer were operated on conservatively and received postoperative breast irradiation (51–56.1 Gy) with two different kinds of boost. One hundred and eighty-eight patients received 12 Gy boost dose with external electron beams after whole-breast irradiation, while 190 patients received 9 Gy boost with intraoperative electrons before whole-breast irradiation. Although not randomized, both groups of treatment were well balanced. After a minimum median follow-up of 50 months, the 5-year actuarial rate of in-breast recurrence was 4.3% (95% CI, 1.9–8.3%) and 0% (95% CI, 0-1.9%), respectively ($p=0.0018$). The ELIOT boost was proved to be not only time-saving but also strongly effective. The same Austrian group from Salzburg did a similar study on patients with locally advanced tumor treated with primary anthracycline-based chemotherapy [62]. Eighty-three patients were given 9 Gy ELIOT boost and 26 patients received postoperative external boost radiotherapy (median dose 12 Gy, range 6–16 Gy) following WBRT. After a minimum median follow-up of 59 months, two recurrences in each groups, all in the original tumor bed, were detected. No statistical difference was found in the actuarial rates for local control, locoregional control, metastasis-free survival, disease-specific survival, and overall survival. Higher local control using the ELIOT boost, although not statistically significant, was also found in the study from Udine [44]. In the population aged <48 years treated with IORT boost and external radiotherapy to the whole breast, no recurrence was seen at 6 years of follow-up, while the group treated with external WBRT presented a cumulative local recurrence rate of 8.3% (95% CI, 0–22.7). At IEO, Milan, the ELIOT boost was implemented for premenopausal patients with early disease and undergoing conservative surgery. Young age is proved to be an independent risk factor for local recurrence, and limiting the irradiated area to the tumor bed only is not considered adequate. With the aim of shortening the duration of treatment without changing the philosophy of the adjuvant approach to treat both the breast and the tumor bed, a hypofractionated scheme was designed at IEO. It consists of 13 fractions over 2.5 weeks (total dose of 37.05 Gy) following the 12 Gy intraoperative boost, and it starts very close to surgery, usually during the third week from the tumor removal, as soon as the wound heals. This short WBRT allows patients to undergo systemic treatment with no interaction with radiotherapy, because the whole treatment is completed 1 month and a half after surgery. The preliminary results on 211 patients were published in 2008 [63]. Acute toxicity was low. Intermediate toxicity was evaluated in 108 patients, with a medium follow-up of 9 months. Only one grade 4 skin toxicity was observed, in an obese woman, who underwent breast remodeling during the chemotherapy course, and one grade 3 skin side effect. For postmenopausal women who present high aggressive tumor features, a shorter hypofractionated scheme, consisting in eight fractions of 4 Gy each delivered over 1 week and a half, using intensity-modulated radiotherapy (IMRT) is currently being investigated at IEO.

56.5.2 The ISIORT-Europe Experience

A joint analysis was carried out by seven RT centers across Europe with the aim of evaluating the effectiveness of

ELIOT boost with the support of ISIORT-Europe. A total of 1109 patients treated with the ELIOT (median dose of 10 Gy) followed by external WBRT with 50–54 Gy entered the joint analysis [64, 65]. At a median follow-up of 72.4 months (0.9–239 months), only 16 local recurrences, equally distributed between true and elsewhere local relapses, were observed, and the local tumor control rate was as high as 99.2%. Analyzing the age as predictor for local failure, the crude annual local relapse rates were 0.64%, 0.34%, 0.21%, and 0.16% in the age groups <40, 41–49, 50–59, and ≥60 years, respectively. This trend toward less local relapse as the age increased was observed for both true and elsewhere recurrences. On multivariate analysis, grade 3 was a significant factor only for true local relapses. At univariate analysis, negative hormonal receptor status and age under 40 proved to be significant factors for local failure. When the time gap between ELIOT boost and WBRT was considered, starting WBRT within 70 days or at more than 140 days after ELIOT had no influence on local control.

On the basis of the positive experiences on ELIOT boost, the HIOB trial, a multicenter prospective one-armed study of hypofractionated whole-breast irradiation following intraoperative electron boost (http://www.clinicaltrials.gov/ct2/show/NCT01343459?term=hiob&rank=1), started in January 2001 on behalf of the ISIORT-Europe [66]. The eligibility criteria included women aged ≥35, with early-stage breast cancer (T1-2, N0-1), any grade, any hormonal receptor status and HER2 status, unifocal or limited multifocal lesion, or free surgical margins. The primary endpoint is to prove the superiority of the experimental scheme in terms of local tumor control by benchmarking with best published results after "gold standard" RT. The HIOB schedule consists of 10 Gy ELIOT boost followed by 40.5 Gy to the whole breast in 15 fractions/3 weeks. The trial is currently open and recruiting patients. As of November 2013, 426 patients from seven radiotherapy centers entered the trial. No major complications were recorded. At a median FU of 13 months, distant metastases in two cases and local relapse in none were observed [67].

56.6 Nipple-Sparing Mastectomy

Nipple-sparing mastectomy (NSM) combines a skin-sparing mastectomy with the preservation of the nipple-areola complex (NAC). In the past, a number of historical publications advised against the use NSM in the invasive setting because of the high rate of nipple involvement reported. Specifically addressed studies in the literature reported NAC involvement ranging from 8% to 33%, with the majority at 25%, but some studies showed nipple involvement rates as high as 58% [68, 69]. Recently, the indications for NSM have been expanded [70].

The approach of combining the surgical technique with ELIOT to the nipple/areola complex was set up at IEO, Milan, in order to kill the potential neoplastic cells in the retroareolar tissue intentionally left by the surgeon behind the areola to preserve the blood supply. The ELIOT schedule consisted of one fraction of 16 Gy at the 90% isodose, which was calculated to be equivalent to 42 Gy given on conventional fractionation [71, 72]. This approach has never met large consensus, and some concerns have arisen about the real effectiveness of this procedure. A report on 1001 NSMs receiving ELIOT to the NAC, in the period 2002–2007 at IEO, Milan, with a median follow-up of 20 months showed promising results and encouraged the experience [73]. NAC necrosis occurred completely in 3.5% and partially in 5.5% of the cases, leading to 5% NAC removal. In 2% of patients infections complicated the postoperative course, and the implant was removed in 4.3% of the cases. No recurrences were observed in the NAC, but 1.4% of the patients experienced local failure on the mastectomy site. In hindsight of a more detailed report on 934 patients, treated at IEO with a median follow-up of 50 months [74], the role of ELIOT on the NAC became uncertain. The proportion of stage II and III disease was as high as 42% and 15%, respectively, and 38% of patients had 1–3 positive lymph nodes. Among 772 invasive cancer patients, the incidence of local recurrence was 3.6% in the mastectomy site and 0.8% on the NAC. Among 162 patients with intraepithelial neoplasia, local recurrence accounts for 4.9% in mastectomy site and 2.9% on the NAC. Considering the histology of the local recurrences on the NAC, seven Paget disease and ductal carcinoma in situ in the underlying ducts and four invasive cancers were detected. All the recurrences were excised. Interestingly in 70 patients with negative frozen section who underwent ELIOT to the NAC, the final definitive histology was positive. Even if the frozen section examination shows high accuracy in predicting the presence of tumor cells in the retroareolar tissue, 8% of false-negative results were observed. In agreement with the patients, the NAC was preserved despite the lack of free margin, and no local relapse was observed with a median follow-up of 50 months. In the IEO series the factors involved in local recurrence on the NAC after invasive cancer were tumor size, receptor status, HER2/neu, grade, and Ki-67 (looking specifically at the NAC relapses, the risk factors were EIC, hormonal receptor, and biomarkers). After ductal carcinoma in situ, prognostic factors for the NAC recurrence were also the young age and retroareolar margins.

The role of ELIOT on the NAC should be demonstrated in controlled clinical trials. The current surgical technique removes almost the entire retroareolar breast tissue and creates thin nipple areolar flap to preserve the subdermal vessels. Besides, most recurrences occur mainly in the mastectomy site rather than from major ducts left behind the nipple. These

observations questioned the use of ELIOT on the NAC, and this approach is being abandoned in favor of more careful clinical and radiological selection of patients.

56.7 Special Conditions

56.7.1 Breast Augmentation

In selected patients having breast augmentation surgery, ELIOT full dose allows avoiding the well-known complications related to external RT, such as capsular contracture, cutaneous fibrosis, and progressive asymmetry, leading to unfavorable aesthetic results [75].

ELIOT full dose can be performed after quadrantectomy with concomitant immediate augmentation mammoplasty only to the tumor bed. By sparing skin and pectoralis major muscle, ELIOT does not cause fibrosis in pectoralis muscle and in the implant/tissue interface or inadequate healing of the skin [76].

56.7.2 Previous Thoracic Radiotherapy

Survivors of Hodgkin's lymphoma carry an increased risk of treatment-related subsequent malignant neoplasms. Breast cancer accounts for more than 40% of the excess risk of a second cancer [77]. When breast RT is indicated as part of breast cancer adjuvant strategy, previous chest irradiation poses serious concerns regarding the risk of overlapping doses to critical organs, such as the heart, lungs, and breasts. In some cases, modified radiation techniques such as the lateral decubitus isocentric position [78] are necessary to protect the underlying heart and lung. ELIOT could represent an option for selected cases, taking advantage of limited radiation fields and of the capability to spare the adjacent organs. Forty-three patients affected by early breast cancer, previously treated with mantle radiation for malignant lymphoma, underwent breast conservative surgery and ELIOT. Median interval between lymphoma and breast cancer occurrence was 19 years. A total dose of 21 Gy (prescribed at 90% isodose) in 39 patients (91%), of 17 Gy (prescribed at Dmax) in 1 patient, and of 18 Gy (prescribed at 90% isodose) was delivered. Good tolerance was observed in all patients. After a median follow-up of 52 months, local recurrence occurred in 9% of the patients and metastases in 7% of the patients [79–81].

56.7.3 Concomitant Systemic Diseases

Patients with severe cardiovascular diseases, respiratory syndromes, skin disorders such as vitiligo, and large pigmented hypertrophic thoracic scars due to skin burns from hot water in childhood might benefit from excluding the skin, heart, and lung from the radiation fields [81]. A dosimetric study evaluating the dose absorbed in the subclavicular region, where cardiac implantable electronical devices (CIED) are usually placed, was performed in healthy patients undergoing BCS and ELIOT [82]. The dose measured with thermoluminescent dosimeters seems to be safe for patients using cardiac devices, since it does not exceed the recommended dose threshold of 2 Gy. Therefore, when clinically indicated, ELIOT might be a valid alternative to external irradiation.

56.7.4 Pregnancy

In vivo dosimetry study was performed at IEO, Milan, Italy, with thermoluminescence radiation detectors (TLDs) placed in three different positions across the abdomen and into the uterus in nonpregnant patients receiving ELIOT with the aim to assess the safety of such treatment in pregnant patients [83]. The embryo/fetus must be deemed sensitive to radiation at all stages of gestation, but most biological effects of radiation have a dose-response relationship. For deterministic effects, such as malformation or mental retardation, a threshold value is approximately 0.1–0.2 Gy [84].

With external breast adjuvant irradiation, the estimated dose to the fetus ranges between 0.14 and 0.18 Gy, doses for which the safety of the embryo/fetus is uncertain [85]. The dosimetry results showed a mean dose of 1.7 mGy in the utero, of 3.7 mGy on subdiaphragm area, and of 0.9 mGy on the pubic region. These findings indicate that ELIOT would be safe for the fetus as doses of a few mGy are not associated with measurable increased risk of fetal damage.

Conclusion

ELIOT represents an attractive option, both for physicians and patients. By reducing the entire radiation treatment of 5–6 weeks from a few fractions up to a single session during surgery, ELIOT has a favorable impact on treatment costs and patients' convenience. One of its strengths is that of potentially decreasing normal tissue toxicity, since the skin, the subcutaneous tissue, and the thoracic wall along with the underlying structures are not irradiated. However, ELIOT suffers from some limitations which must be overcome to improve the efficacy and the cost-effectiveness. The proper identification of the ideal candidates is challenging because at the time of the intraoperative irradiation, the complete tumor profile is not available. Efforts must be made in order to obtain the biologic and histologic tumor characterization through preoperative (true cut or core biopsy) and intraoperative (frozen sections) pathologic assessment. Subgroup analysis showed that ELIOT is effective in selected patients for whom external whole-breast radiotherapy would not be more

beneficial. Other issues address the proper width of the intraoperative radiation field to uniformly cover all the area at risk of microscopic disease. Besides, the appropriateness of 21 Gy full dose is a matter of debate. The linear-quadratic model used to derive the biological equivalent dose seems not adequate in the range of high dose/fraction. The ELIOT boost dose can be successfully integrated in the schedules of adjuvant breast radiotherapy.

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Radiotherapy for Metastatic Lesions

57

Per Karlsson and Dan Lundstedt

57.1 Introduction

Radiotherapy of metastatic lesions is generally of palliative intent. In these situations it is important to weigh the symptom-relieving effects against potential side effects of the radiotherapy. Metastatic breast cancer includes a wide range of clinical situations, all the way from situations in which the patient with modern treatment can be expected to live for many years to symptom-relieving situations in the end of life. The radiotherapy must be adjusted to the overall situation for the patient. The term oligometastatic disease, first described by Hellman and Weichselbaum [1], means a limited spread of the disease with just a few metastases but not a fully disseminated disease.

In patients with oligometastatic disease, the modern systemic treatments and the possibilities with the new techniques in the radiotherapy to deliver high doses with sharp dose margins have actualized the question if not some of these patients may have a curative potential [2]. At least, there are some long-term survivors in series with patients treated with radiotherapy with oligometastatic disease [3]. When it comes to radiotherapy in patients with metastatic lesions, it is important to include the total situation for the patient in a multidisciplinary dialogue. For some patients late in life, it may be wise to refrain from radiotherapy or just give a single pain-relieving fraction, and for others the decision may be to choose an extensive stereotactic radiotherapy if a potential for long-term survival exists.

57.2 Bone Metastases

In 2012 Chow et al. published meta-analyses of 25 randomized radiotherapy trials which compared a single fraction with multiple fractions for pain relief in patients with bone metastases [4]. The overall response rate after a single dose was 60% and after multiple fractions 61%. Complete pain response was found in 23% after a single fraction and 24% after multiple fractions [4]. However, the re-treatment rate was higher after single fraction (20%) than after multiple fractions (8%). This review confirms the results from previous reviews that a single dose is as effective as multiple fractions [5, 6]. Hartsell et al. published a randomized phase 3 study in patients with bone metastases specifically from breast and prostate cancer and compared a single dose of 8 Gy versus multiple fractionations of 3–30 Gy [7]. A single dose of 8 Gy was as effective as multiple fractionations. There was less acute toxicity after 8 Gy, 10% compared to 17% after 30 Gy. The frequency of pathologic fractures within the treatment field was similar between the groups, 4% and 5%, respectively. However, the re-treatment rate was higher after 8 Gy, 18% compared to 9% after 30 Gy. Also the guidelines for palliative radiotherapy for bone metastases from the American Society for Radiation Oncology (ASTRO) state that a single fraction is as effective in pain relief as multiple fractions and that a single fraction generally is more convenient for the patients and caregivers [8].

Dennis et al. reviewed various doses of single fractions for the treatment of painful bone metastases and found that 8 Gy probably is close to an optimal dose [9]. Wong et al. made a systemic review of re-irradiation for painful bone metastases and found that the overall and complete response rate was 68% and 20%, respectively, which is comparable to the response frequencies seen after the initial radiation for bone metastases [10]. To reduce the acute pain flare seen in some patients after palliative irradiation of bone metastases, Chow et al. showed in a randomized study that taking 8 mg dexamethasone at the day of the radiotherapy and during the following 4 days reduced the frequency of patients having the pain flare from 36% to 26% [11] (Fig. 57.1).

57.3 Malignant Spinal Cord Compression

Malignant spinal cord compression (MSCC) is a feared complication to metastatic cancer. A population-based study from Ontario reported that about 2.5% of all metastatic

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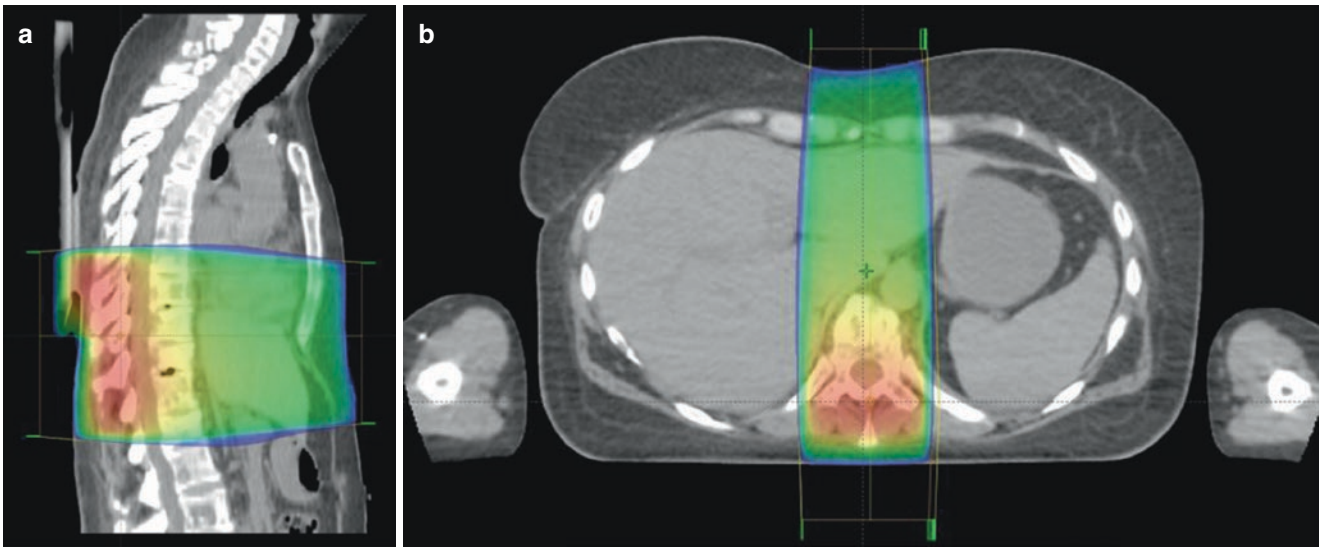

Fig. 57.1 Palliative radiotherapy for part of the thoracic spine. Sagittal (**a**) and transversal (**b**) views of a treatment plan with a dose prescription of 8.0 Gy in a single fraction. The *blue color* corresponds to the 50% dose level. Eclipse™ Treatment Planning System

patients dying from cancer had at least one admission to a hospital with MSCC [12]. Patchell et al. studied the effect of decompressive surgery in addition to radiotherapy in a randomized study in which the patients had either decompressive surgery followed by radiotherapy (n = 50) or radiotherapy alone (n = 51) [13]. The radiotherapy was given with ten 3 Gy fractions, and dexamethasone was given in both arms. The primary endpoint was ability to walk after treatment. For patients having surgery plus radiotherapy, 84% were able to walk after treatment in comparison to 54% of the patients having radiotherapy alone. A later publication from the same group indicated that the additional benefit of the decompressive surgery only was seen in the age group younger than 65 years [14]. In patients fit for surgery and a single-level MSCC decompressive surgery followed by radiotherapy is recommended.

For older patients and patients not fit for surgery, radiotherapy alone is recommended [15]. Two randomized Italian trials comparing different fractionation schedules in patients with short life expectancy could not find any significant difference in outcome [16, 17]. In the first trial, two 8 Gy fractions were compared with a split course of three fractions of 5 Gy followed by five fractions of 3 Gy [16], and 68 and 71% of the patients were able to walk after the two radiotherapy regimens, respectively. In the second trial, 8 Gy as a single fraction was compared to two 8 Gy fractions, with 62% and 69% of the patients were keeping or improving their walking function in the two fractionation arms, respectively [17]. This difference was not statistically significant. Thus in patients with short life expectancy, 8 Gy as a single fraction is recommended. Patients with MSCC are generally recommended steroids, if not medically contraindicated [15].

57.4 Brain Metastases

In early breast cancer patient randomized in different clinical studies (n = 9524) within the International Breast Cancer Study Group (IBCSG), the 10-year incidence of brain metastases was 5.2% [18]. Among patients with metastatic breast cancer, approximately 10–16% will develop brain metastases [19]. The choice of treatment depends on various factors, e.g., number of metastases and the overall prognosis. The Radiation Therapy Oncology Group (RTOG) has from their trials established three prognostic groups using the recursive partitioning analysis (RPA) [20, 21]. The RPA classes were based on Karnofsky performance status, age, primary tumor status, and presence of extracranial metastases. The RPA has been challenged by the graded prognostic assessment (GPA) index, which also includes number of brain metastases [22], and further prognostic information have been derived in the diagnosis-specific GPA which also included information of the primary tumor [23]. For breast cancer patients with brain metastases, the breast-GPA has been established which takes breast cancer subtype, age, and Karnofsky performance index into consideration [24]. The breast-GPA score is the sum of the score for these three factors (Table 57.1). Sperduto et al. have shown in a retrospective multi-institutional database study of 400 breast cancer patient with brain metastases that total scores of 0–1, 1.5–2, 2.5–3, and 3.5–4 correspond to median survival times of 3.4, 7.7, 15.1, and 25.3 months, respectively [24].

Looking at different subtypes of the breast cancer only, the mean survival time after diagnoses of brain metastases was 7.3, 10.0, 17.9, and 22.9 months for women with triple negative, hormone receptor positive/HER2 negative, hormone receptor negative/HER2 positive, and hormone receptor positive/HER2 positive, respectively [25].

57.4.1 Whole-Brain Radiotherapy (WBRT)

The purpose of WBRT is to irradiate both visual tumors and microscopic disease (Fig. 57.2). Whole-brain radiotherapy is generally recommended in patients with multiple brain metastases or patients with poor life expectancy. If life expectancy is very short, less than 3 months, supportive care alone with dexamethasone may be recommended [26]. One old randomized trial included 48 patients with brain metastases and compared the effect of WBRT + prednisone with prednisone alone and found an increase in survival from 10 to 14 weeks in favor of the group receiving the combination [27]. In a newly presented abstract from ASCO, 538 non-small cell lung cancer patients with brain metastases were randomized to 20 Gy in five fractions or optimal supportive care only [28]. No significant difference in overall survival (65 days vs. 57 days) or quality-adjusted survival was seen in this patient group. The standard fractionation schedules are ten times 3 Gy in 2 weeks or 20 Gy in four or five daily fractions. Davey et al. compared a higher total dose of 40 Gy (20 × 2 Gy) with 20 Gy (5 × 4 Gy), but the median survival time was 19.1 weeks in both arms [29]. Also Graham compared a higher total dose 20 × 2 Gy, twice daily, to 4 × 5 Gy, which resulted in similar median survival, 6.1 and 6.6 months, respectively [30]. In a Cohrane report from 2012, the authors concluded no other dose fractionation schemes thus far compared to 30 Gy in ten fractions or 20 Gy in 4–5 fractions resulted in any benefit in overall survival, neurologic function, or symptom control [31]. When interpreting the length of the survival time in the abovementioned studies, one has to remember that these studies contained mainly lung cancer patients and only a smaller fraction had breast cancer as the primary tumor. Thus the breast cancer-graded prognostic assessment index (breast-GPA) must be considered while estimating life expectancy after WBRT in breast cancer patient with brain metastases [24]. WBRT can cause chronic neurocognitive side effects as affected memory function [32] which becomes especially important among long-time survivors.

Table 57.1 The graded prognostic assessment for breast cancer (breast-GPA) [24]

Score					
Factor	0.0	0.5	1.0	1.5	2.0
Age (years)	≥60	<60	–	–	–
KPS	≤50	60	70–80	90–100	–
Genetic subtype	Her2 neg and ER/PR neg	–	Her2 neg and ER/PR pos	HER2 pos and ER/PR neg	HER2 pos and ER/PR pos

KPS Karnofsky performance status

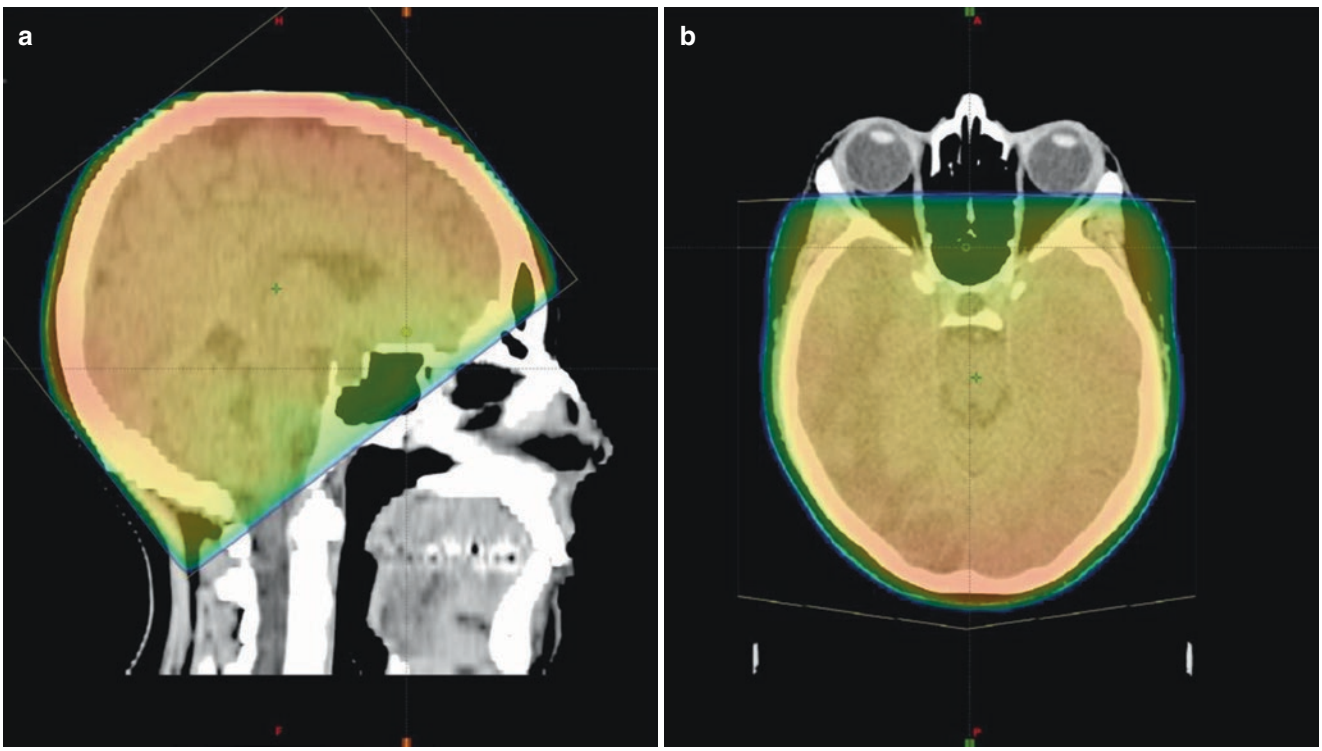

Fig. 57.2 Whole-brain radiotherapy (WBRT) with two opposing fields. Sagittal (**a**) and transversal (**b**) views of the treatment plan with a dose prescription of 4.0–20.0 Gy. The *blue color* corresponds to the 50% dose level. Eclipse™ Treatment Planning System

57.4.2 Stereotactic Radiotherapy for Brain Metastasis

Stereotactic radiosurgery (SRS), stereotactic radiotherapy (SRT), and Gamma Knife all refer to treatment techniques where a group of convergent beams is used to target an exactly defined lesion. This technique makes it possible to treat local brain metastases with a very high dose and at the same time spare the normal brain tissue. Single doses of at least 20 Gy seem to result in better local control rates [33], but the maximum tolerated dose is dependent on the maximum diameter of the lesion [34] (Fig. 57.3).

Stereotactic radiotherapy can be used alone or in combination with WBRT. In the RTOG 9508 trial, 333 patients with 1–3 brain metastases were randomized to WBRT with or without stereotactic radiotherapy [35]. For patients with one brain metastasis, stereotactic radiotherapy improved the median survival from 4.9 months to 6.5 months, $p = 0.01$ [35]. No survival benefit was seen for those with two or three metastases [35].

The addition of WBRT to SRS or surgery does not affect survival, but it increases the total intracranial tumor control by reducing the number of new brain metastases and by improving the local control [36]. The EORTC 22952-26001 study of SRS or surgery with or without WBRT for patients with 1–3 metastases found that WBRT reduced the 2-year relapse rate both at the initial sites (radiosurgery, 31–19%; surgery, 59–27%) and at new sites (radiosurgery, 48–33%; surgery, 42–23%). However, the median overall survival time was similar in the both arms, 10.9 months with WBRT and 10.7 without ($p = 0.89$). The median time to impaired functional status, WHO performance status more than two, was 10.0 months without WBRT and 9.5 months with WBRT ($p = 0.71$), indicating that WBRT did not prolong the duration of functional independence [36]. According to a Cochrane review 2014 including five randomized controlled trials, adding WBRT to SRS or surgery among patients with 1–4 metastases decreased the relative risk of any intracranial disease progression at 1 year by 53% (RR 0.47, 95% CI 0.34–0.66), but there was no clear evidence of a difference in OS (HR 1.11, 95% CI 0.83–1.48) or in PFS (HR 0.76, 95% CI 0.53–1.10) [37].

Chang et al. studied the effect on neurocognitive function in patients with 1–3 brain metastases randomized to stereotactic radiotherapy with or without WBRT [32]. The study was stopped by the data monitoring committee, already after 58 included patients, since the probability of decline in memory function at 4 months was much higher in the group having WBRT, 52% versus 24% [32]. To avoid the side effect

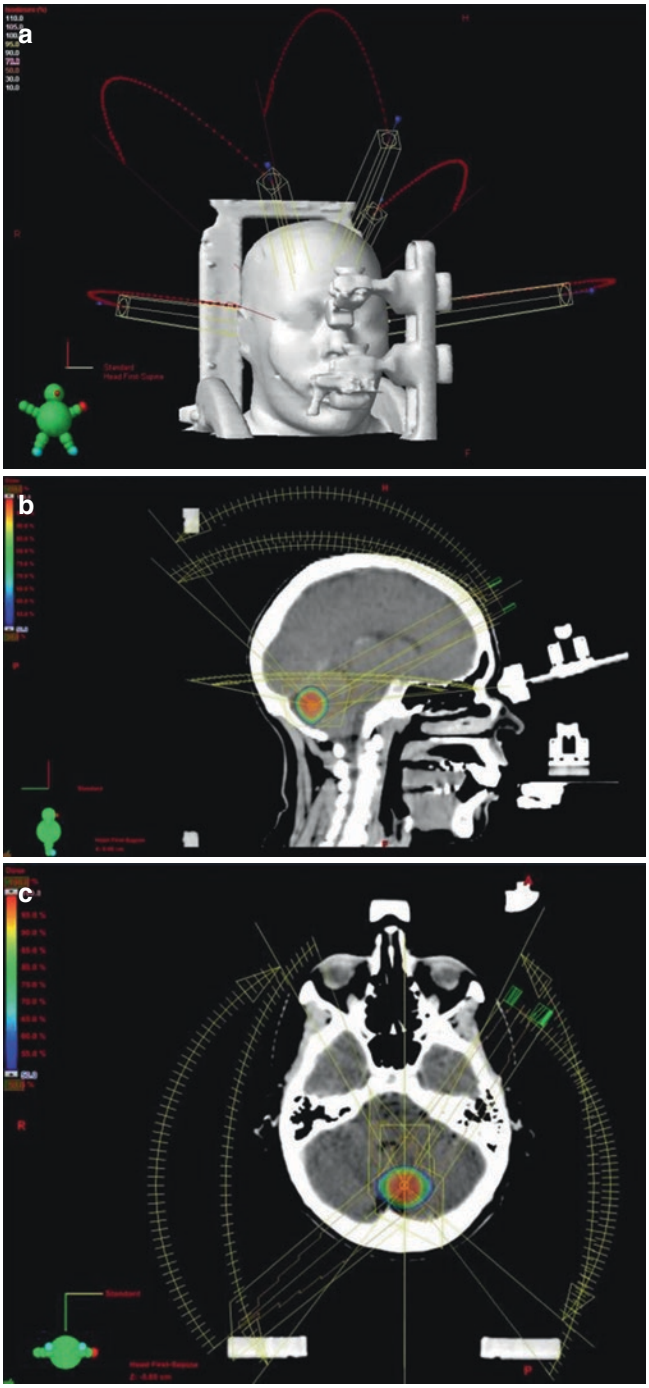

Fig. 57.3 Stereotactic radiotherapy plan of a single metastasis in the cerebellum with a dose prescription of 20.0 Gy in one fraction. The DRR (**a**) with the immobilization system (trUpoint ARCH™ SRS/SRT System: head support, bite tray, mask, and nasion cushion) as well as the treatment archs (Varian TrueBeam™ STx). Sagittal (**b**) and transversal (**c**) views. The *blue color* corresponds to the 50% dose level. Eclipse™ Treatment Planning System

related to WBRT, the authors recommend initial treatment with SRS followed by close monitoring [32], which has also been supported by the American College of Radiology [38].

In the NCCTG N0574 (Alliance) study, 213 patients with 1–3 brain metastases were randomized to receive or not to receive WBRT in addition to SRS with cognitive progression as primary endpoint [39]. The study was presented at ASCO in 2015. They showed that cognitive progression at 3 months was more frequent after WBRT + SRS vs. SRS alone (91.7% vs. 63.5%, respectively, $p = 0.007$) and at 6 months there was still a significant difference (97.9% vs. 77.8%). The difference in cognitive progression seen was mainly due to impairment in immediate and delayed recall as well as impairment in verbal fluency. To avoid the early side effects related to WBRT in combination with SRS, the author recommended initial treatment with SRS alone followed by close monitoring [39].

57.4.3 New Techniques to Spare Cognitive Functions

The radiation-induced cognitive impairment seems to be associated with exposure to the hippocampus region [40] and especially the neural stem cells located in the hippocampal dentate gyrus [41]. Therefore, radiotherapy technique to minimize the dose to the hippocampal region has been developed [42]. In the RTOG 0933 trial, which was a phase II single-arm study, 113 patients with brain metastases were treated with IMRT (intensity-modulated radiotherapy) to the whole brain but avoiding the hippocampal region [43]. The primary endpoint was decline in delayed recall at 4 months. The relative decline seen among 48 evaluable patients was 7%, which was lower than 30% seen in historical controls of WBRT [43]. Further neuroprotective drugs have been tested. Memantine, an N-methyl-D-aspartate (NMDA) receptor agonist, has been tested during WBRT with some positive indications on delayed recall, however not statistically significant [44].

57.5 Extracranial Oligometastases

Oligometastatic disease is a clinical situation with a few metastases, but not yet with disseminated spread, first described by Hellman and Weichselbaum [1]. In the same manner as surgical removal of liver metastases in colonic cancer [45] or removal of lung metastases in a sarcoma patients [46] has resulted in long-term survivors, one may expect some long-term survivors after SBRT in patients with oligometastatic disease. The question about possibilities of stereotactic body radiation therapy (SBRT) in metastatic breast cancer has been more actualized with the improvement of the systemic therapies in controlling the spread of the disease. Several prospective series with stereotactic body radiation therapy have proven high local control rates in the treated metastases (~80%) and a progression-free survival of about 20–30% [47].

57.5.1 Stereotactic Body Radiation Therapy (SBRT)

The techniques to exactly deliver the dose to the target volumes have developed remarkably during later years. American Society for Therapeutic Radiology and Oncology (ASTRO) describes stereotactic body radiation therapy (SBRT) in the following way: “Stereotactic body radiation therapy (SBRT) is an external beam radiation therapy method used to very precisely deliver a high dose of radiation to an extracranial target within the body, using either a single dose or a small number of fractions” [48]. SBRT is suitable for small lesions with a largest dimension of about 4–5 cm. For larger lesions the radiation volumes increase rapidly since the volume of a sphere multiplies by a factor three times the radius (Fig. 57.4).

SBRT is of special interest in patients with oligometastatic disease. One of the larger series has been published by Milano and colleagues [3, 49]. They present a follow-up of 121 patients with five or fewer metastases treated with SBRT. The median number of treated lesions was 2, and the median sum of the gross target volume (GTV) was 28 cm^3. For liver and lung metastases, the most common used fractionation was 5 Gy times ten fractions. The 2-, 4-, and 6-year overall survival (OS) rates were 50%, 28%, and 20%, and the 2-, 4-, and 6-year freedom from widespread distant metastasis (FFDM) rates were 35%, 26%, and 21%. For the 39 patients with breast cancer as their primary tumor, the outcome was better, 74%, 54%, and 47% for 2-, 4-, and 6-year OS and 52%, 43%, and 36% for FFDM at 2, 4, and 6 years. For the breast cancer patients responding to the systemic treatment before the SBRT, the 2-year OS was 81% in comparison to 55% for those not responding to systemic therapy before SBRT.

Andratschke et al. found that the size of the gross target volume (GTV) was correlated to inferior OS and the biologic equivalent dose was correlated to local control in a series of 74 patients with one to four liver metastases treated with SBRT in three to five fractions of 5–12.5 Gy per fraction [50].

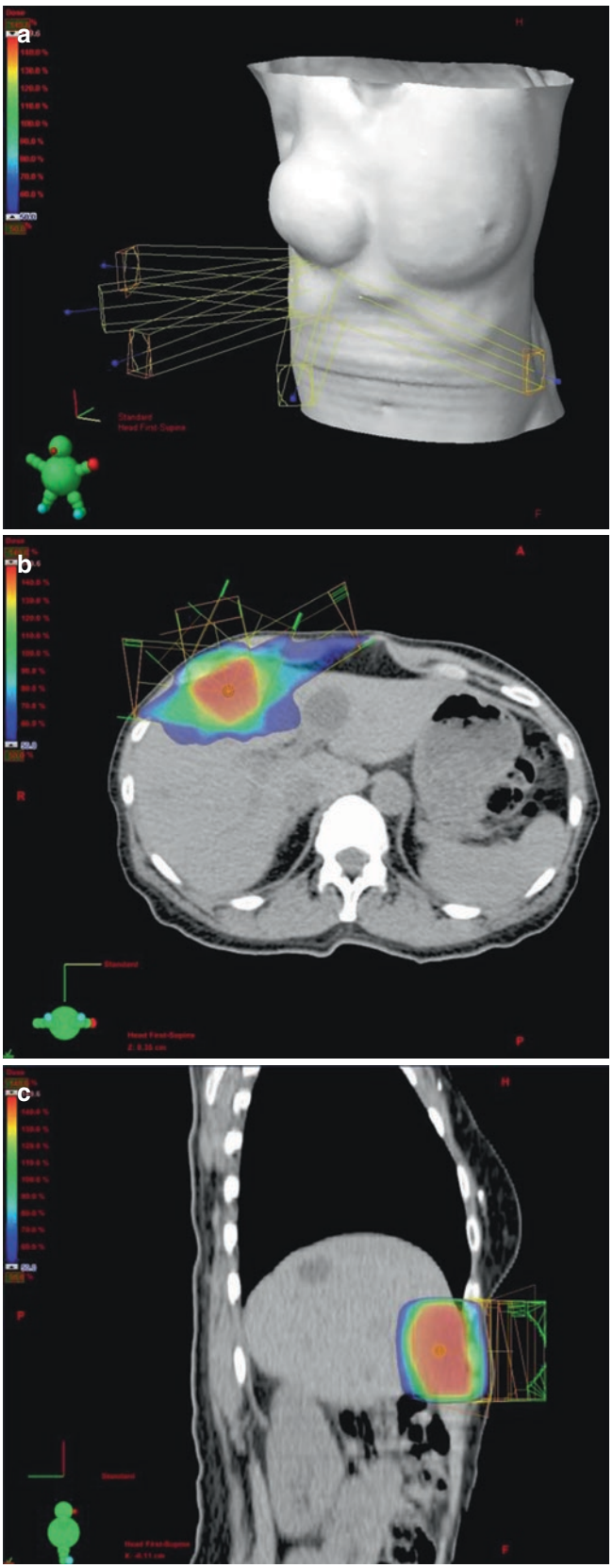

Fig. 57.4 Stereotactic body radiotherapy plan of a liver metastasis with a dose prescription of 40.0 Gy in four fractions. The target definition is based on the movements captured with a 4D CT. The DRR (**a**) with the treatment fields. Transversal (**b**) and sagittal (**c**) views. The *blue color* corresponds to the 50% dose level. Eclipse™ Treatment Planning System

To conclude, SBRT gives possibilities to long-term survival in some patients with oligometastatic disease. For breast cancer patients previously responding to systemic therapy and with controlled disease outside the oligometastatic lesions, SBRT is a reasonable option after discussion in a multidisciplinary team. Unfortunately we lack evidence from randomized controlled trials, and to exactly know the indication for SBRT, randomized controlled trials are warranted.

57.6 Locally Advanced and Recurrent Breast Cancer

The role of radiotherapy in locally recurrent breast cancer depends on the extent of the recurrence, the sensitivity to systemic therapies, the symptoms of the patient, and previous radiation within the area. In patients refractory to systemic therapies, the aim of the radiotherapy is to achieve local control and to diminish symptoms. Bedwinek and colleagues found that the dose needed for local control was dependent on the size of the recurrence [51]. Their study indicated the need of 50 Gy in 2 Gy fractions if the recurrence was completely excised, 55 Gy if remaining tumor was <1 cm, and 60 Gy if remaining tumor was 1–3 cm. If remaining tumor was more than 3 cm, 65 Gy was not sufficient. Another nonrandomized series with primary radiotherapy in 192 patients with advanced breast cancer have shown better 5-year local control rates with doses greater than 60 Gy [52].

For patients not previously irradiated and with longer expected survival, a radiotherapy series with smaller fractions to 50–60 Gy may be indicated. For patient with shorter life expectancy and local symptoms, a short course of 20–30 Gy in 5–10 fractions may be more appropriate [53].

Recurrence in a previously irradiated volume is a complex clinical situation, and the potential benefits and harms of reirradiation must be weighed carefully in a multidisciplinary conference. The risk of fibrosis, necrosis, brachial plexopathy, and rib fractures depends on the combined dose and the volume exposed [54, 55]. There are small series of reirradiation with total combined doses up to 100 Gy with local control rates at 1 year of about 60–80% and with limited short-term toxicity [56, 57]. Hyperthermia seems to potentiate the effect of the reirradiation [56, 58]. Electrochemotherapy (ECT) may be an alternative and complementary local treatment for cutaneous metastases [59].

57.7 Integration with Systemic Therapy

Regarding bisphosphonates and palliative radiotherapy, there are some indications of possible additive effects when these treatments are given during the same period of time [60–62], even if no formal randomized studies of sequential and concurrent use have been performed.

For endocrine therapy it is reasonable to continue with the endocrine treatment during the palliative radiation. At least in early breast cancer, no significant difference was seen in studies with concurrent or sequential use of tamoxifen and radiotherapy [63, 64]. Regarding chemotherapy the palliative radiotherapy is often given in between the chemo courses and not concurrently. Some studies in locally advanced breast cancer have studied the potential benefit of concurrent radiochemotherapy but not much data exist [65–67]. There are also some indications of increased toxicity in these trials which must be weighed against potential benefits in a palliative situation.

57.7.1 Radiation Recall

First to describe radiation recall was D'Angio [68], who already in 1959 described reactivation of latent radiation effects in the skin when actinomycin D was given weeks after radiotherapy. The reaction was sharply restricted to the area of the previous radiation.

Many case reports of radiation recall exist but systematic series are rarely reported. Kodym et al. followed 142 patients having palliative radiotherapy, and of these 91 received subsequent palliative chemotherapy within 6 months [69]. Of these 91, 8 patients (8.8%) did show a reactivation of the skin reaction within the former radiation field (radiation recall) [69]. The skin reactions ranged from dermatitis, WHO grade I, to exfoliative dermatitis, WHO grade III. The reaction was seen after several types of chemo, taxanes, alkylating agents, antimetabolites, and antitumor antibiotics. During later years radiation recall has been actualized when introducing modern targeted therapies, for instance, after treatment in melanoma with the BRAF inhibitor vemurafenib [70]. When introducing new targeted therapies, some awareness among doctors of these types of reactions should be warranted.

Conclusions

Radiotherapy for metastatic lesions includes a wide variety of clinical situations from symptom control late in life to potentially curative advanced stereotactic radiotherapy in oligometastatic disease. The combination of the modern imaging techniques and the extremely high precision of dose delivery has made it possible to increase the doses to the metastatic lesion but still spare the surrounding normal tissues. With new possibilities in radiotherapy, it is even more important to discuss what is appropriate with the patient and in a multidisciplinary team. Late in life it is important with symptom control and not to overtreat the patient with advanced technology; however with long life expectancy and with appropriate systemic treatment, it is also important to really use the advanced radiotherapy techniques if considered right for the patient after a multidisciplinary dialogue.

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Part IX

Special Conditions Requiring Multidisciplinary Approaches

Emergencies in Breast Cancer

58

Lorenzo Gianni, Maria Vittoria Stefania Nicoletti, and Valentina Arcangeli

58.1 Introduction

The increasing number of cancer survivors [1] and of patients living with a "chronic" form of advanced disease, often managed as outpatients, explains their growing request for clinical evaluation at the Emergency Department [2]. Cancer patients may be admitted either for the first symptoms of the disease, for a tumor or treatment-related complication, and for other comorbidities or symptoms near the end of life, sometimes with the characteristics of an oncological emergency (OE) [3]. These are serious and potentially life-threatening complications of cancer that may occur in any phase of the disease. Proper management requires adequate training, integration between different specialists, and timely access to diagnostic and treatment resources. Two major principal groups of OEs are identified: mechanical and metabolic OEs (Table 58.1); other two categories such as hematologic emergencies and treatment-related emergencies are occasionally added [4].

Table 58.1 List of the principal oncological emergencies

Obstructive/structural emergencies	Increased intracranial pressure
	Malignant pericardial effusion
	Malignant spinal cord compression
	Superior vena cava syndrome (SVCS)
Metabolic emergencies	Hypercalcemia
	Hyponatremia and SIADH
	Tumor lysis syndrome
Hematologic emergencies	Disseminated intravascular coagulation (DIC)
Treatment related	Febrile neutropenia

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58.2 Mechanical Emergencies

58.2.1 Increased Intracranial Pressure

Increased intracranial pressure (ICP) is defined as a sustained ICP of more than 20 mmHg (normal values 0–10 mmHg) and is originated by several causes such as space-occupying lesions, edema, and hydrocephalus [5]. Intracranial neoplasm, more often of metastatic origin from lung cancer (20%), renal cancer (10%), melanoma (7%), breast cancer (BC) (5%), and colorectal cancer (1%), is an important cause of ICP [4].

Pathophysiology. Brain metastases (BM) grow after hematogenous spread with predilection for the cerebral hemispheres, cerebellum, and brainstem [6]. The tumor mass effect and the cerebral edema related to blood barrier disruption contribute to the elevation of ICP [7]. The brain, together with cerebrospinal fluid and blood, is contained within a rigid skull, with limited capabilities of compensation for increases in ICP. Large volume changes may result in cerebral herniation or displacement, compression of brain structures, and blood flow impairment with neurological impairment and severe symptoms [5].

Clinical Feature. The most frequent clinical manifestations are headache, nausea, vomiting, and focal neurological signs depending on lesion site or complication. As ICP increases, seizures, altered mental status, and coma may appear; hypertension, bradycardia, and irregular breathing (Cushing triad), diplopia, and pupillary changes may suggest impending herniation [5].

Diagnosis. Contrast-enhanced computed tomography (CT) is the screening test of choice when BM are suspected; non-contrast CT scan may be preferred in more acute clinical condition, when hemorrhage or hydrocephalus is suspected. Contrast-enhanced magnetic resonance imaging (MRI) is the more sensitive diagnostic tool, particularly for metastasis in the posterior fossa [4].

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Management. Nursing considerations include 30° elevation of the head of bed and maintenance of normal body temperature and of blood pressure above 90 mmHg [5]. Hypotonic saline should be avoided. Corticosteroids may reduce capillary permeability. Dexamethasone, the preferred agent, is administered intravenously (IV) or orally, at the dose of 4 mg every 6 h (often after a loading dose of 10–24 mg), even if no general consensus about dose exists [4, 7]. Subsequent dose tapering to the minimum effective amount is indicated. *Pneumocystis jirovecii* prophylaxis should be considered when extended treatment is planned [8]. Mannitol may temporarily be used to reduce ICP, but rebound increase of ICP may occur [9]. The routine prophylactic use of antiepileptic drugs for adult with BM who have not experienced seizures is not recommended [10]. The presence of BM is not viewed as an absolute contraindication to the use of low molecular weight heparin for venous thromboembolic complications [11, 12] that can occur in approximately 20% of patients [13].

Local and systemic treatment of brain metastases may be indicated: in general whole-brain radiation therapy, most commonly used in patients with multiple brain lesions, may prolong median survival from 1 to 2 months to 3–6 months [7].

58.2.2 Malignant Pericardial Effusion

Cancer explains about 10–25% of incidentally discovered pericardial effusion (PE) in asymptomatic patients [14] and even more in symptomatic patients [15, 16]. Pericardial infiltration or metastases from lung cancer (34.4%), BC (16.7%), leukemia (9.4%), and other cancers are the main causes [15]. Inner quadrant-located BC has a fourfold increased risk [17]. Despite a reported poor prognosis for malignant PE [15], no difference in survival was observed in BC patients compared to those with metastatic disease in general [17].

Pathophysiology. Increase of intrapericardial pressure may lead to impaired filling of one or both ventricles and decreased cardiac output resulting in cardiac tamponade, depending on the amount of pericardial fluid and the speed of its accumulation [18].

Clinical Features. Pericardial effusion can be initially asymptomatic; symptoms include dyspnea, chest pain, and/or fullness, cough, weakness, fatigue, anorexia, and palpitations, nausea, dysphagia, hoarseness, and hiccups. Clinical findings include hypotension, increased jugular venous pressure with neck vein distention, and small and distant heart sounds on cardiac auscultation (Beck triad), tachycardia, and pulsus paradoxus [14].

Diagnosis. Routine blood tests, including markers of inflammation and troponins, are recommended in all cases of suspected pericarditis. Low electric voltage or electrical alternans may be evident on electrocardiography registration. Chest x-rays may show cardiomegaly, but transthoracic echocardiography is the imaging test of choice: it detects pericardial effusion and cardiac tamponade and gives important information about ventricular function [14, 18]. A small PE is an echo-free space of <10 mm generally located posteriorly, adjacent to the right atrium; moderate (10–20 mm) and large (>20 mm) PEs tend to become circumferential [19]. Chest CT and MRI allow an accurate study of the chest and pericardium. Cytological or histological diagnosis confirmation may rule out nonmalignant complication [14].

Management. Most of evidence relies on retrospective case series with mixed types of cancer. In case of cardiac tamponade, fluid infusion may be indicated in hypovolemic patients, but elective treatment is ultrasound (US)-guided pericardiocentesis and indwelling catheter placement [6]. Intrapericardial (IP) instillation of sclerosing or cytotoxic agents may be used to prevent PE reappearance. Cisplatin and thiotepa are the agents of choice for IP treatment (CHT), respectively, in case of pericardial involvement by lung cancer and BC [14], with PE control in 83–100% of cases [20–25]. Possible effective alternatives are mitoxantrone, mitomycin-C, bleomycin, and ^{32}P-colloid [26–29]. Surgical approach with pericardial window and pericardiodesis or percutaneous balloon pericardiotomy are alternatives for severe, recurrent, life-threatening PE [30]. Control of the underlying neoplasm is the only significant factor influencing survival [31].

58.2.3 Malignant Spinal Cord Compression

Malignant spinal cord compression (MSCC) is one of the most serious complications of cancer [32]. About 2.5% of patients with advanced cancer develop MSCC [33]. Up to 20% of cases occurs at cancer diagnosis (12% in BC) [34]. Between 5% and 20% of patients with spinal metastases (SM) develop MSCC during the course of their disease [35]. In BC patients with SM, neurological deficit leading to the diagnosis of SM was reported in 1.4% of patients, while 2.8% developed MSCC during follow-up [36].

Pathophysiology. Spinal epidural metastases or locally advanced cancer may compress, displace, or encase the thecal sac that surrounds the spinal cord or cauda equina. Compression may originate by posterior extension of a vertebral body mass or vertebral body collapse and retropulsion of bony fragments into the epidural space, by anterior expansion of a mass arising from the dorsal elements, or by tumoral invasion of the vertebral foramen [32]. About 60–80% of spinal extradural metastasis occurs in the thoracic spine, 15–30% occurs in the lumbosacral region, and less than 10% involves the cervical spine. Up to 50% of patients have involvement of more than one area of the spine [34]. The

damage to the spinal cord is the result of many factors including vasogenic edema, ischemia, venous congestion, and demyelination.

Clinical Features. Back pain is the most frequent symptom of presentation. In a prospective study, 94% of patients reported pain that had been present for approximately 3 months. Most patients (85%) had noticed weakness (median duration of 20 days), difficulty walking, or falls [37]; however, motor symptoms may appear suddenly. Paresthesia, decreased sensation, and numbness are also common. Symptoms related to autonomic dysfunction with urinary retention, incontinence or constipation, and impotence are a later consequence [32].

Compression of cauda equina may be suggested by low back pain; motor symptoms; saddle anesthesia or numbness in the buttocks, thighs, and perineum; urinary retention; overflow incontinence, occasionally with loss of anal sphincter tone; or constipation [38].

Diagnosis. Patients with recent onset of back pain, symmetric weakness, or paresthesia must be quickly evaluated, to prevent significant morbidity and permanent disability. The entire spine should be evaluated to rule out multiple epidural lesions [4]. Plain radiographs may be falsely negative [32]; MRI is the gold standard imaging approach, while CT may be useful when MRI is contraindicated and for surgery and radiotherapy planning [32, 39].

Management. Corticosteroids are indicated to reduce edema, to limit neurological impairment, and to improve pain control [40]. Patients receiving radiotherapy (RT) for MSCC have better outcome if they receive high dose of dexamethasone [41], though controversy exists regarding the optimal dose [39].

Surgery is the treatment of choice for selected patients with good prognosis, unstable spine, compression due to bony fragments, and impending neurologic deterioration, who are unlikely to respond to RT or at risk of spinal damage due to previous radiotherapy [32]. Surgery followed by RT is better than RT alone in preserving walking function [42]. Timing of the procedure is a critical point to attain neurological function preservation. Complete paraplegia, poor prognosis, or frailty contraindicates surgery.

Radiotherapy is indicated for patients not eligible for surgery or as a completion of operation [40, 43]. Hypofractionated RT schedules are effective with acceptable toxicity [44]. A single RT fraction of 8 Gy is recommended for patients with poor prognosis, otherwise a radiation dose of 30 Gy in ten fractions may be considered [44]. Timing of RT and cancer radiosensitivity are critical factors. Spinal stereotactic radiosurgery and intensity-modulated RT may allow more accurate RT delivery to the target [45].

When surgery is not indicated, vertebroplasty and kyphoplasty are options for intractable pain due to pathologic vertebral body fractures [45].

58.2.4 Superior Vena Cava Syndrome

Malignancy represents the main cause of superior vena cava syndrome (SVCS) (60–85%), followed by iatrogenic complication, related to intravascular devices and other benign diseases [44, 45]. Non-small cell lung cancer (50%), small cell lung cancer (22%), lymphoma (12%), metastatic lesions (9%, mainly from BC), germ cell cancer (3%), thymoma (2%), and mesothelioma (1%) are the most frequent causes [46].

Pathophysiology. Obstruction of the superior vena cava (SVC) can be caused by compression or infiltration by an adjacent pathologic process involving the middle or anterior mediastinum and/or by thrombosis within the SVC [6]. The azygos venous system represents the most important collateral pathways, but in case of obstruction at the level of this vein, collateral veins on the chest and abdominal walls may drain the blood toward the inferior vena cava [47].

Clinical Features. Clinical manifestation includes dyspnea; cough; swelling of the face, neck, and arms; cyanosis; and distended neck and chest veins [46, 48]. Pleural effusion may be present in 60% of patients [49]. Edema may compromise the function of the larynx or pharynx, causing stridor, cough, hoarseness, and dysphagia. Neurologic symptoms related to cerebral edema, with headaches, confusion, and significant respiratory impairment, may portend a more serious clinical picture [47]. An SVCS grading system based on clinical findings has been proposed to evaluate the severity of symptoms and the urgency of intervention [49].

Diagnosis. Chest radiography can reveal a mediastinal enlargement, but multi-detector CT with multiplanar reconstructions is the preferred imaging modality [6, 50]. It may provide important information about SVC obstruction, its underlying cause, and collateral circulation [51]. Venography, once the gold standard [52], is performed only when stent placement or surgery is planned [46]; MRI has high sensitivity and specificity and is preferred in case of allergy to radiographic contrast media [52]. ^{18}Fluorodeoxyglucose positron emission tomography may add some details and information useful for prognosis and treatment response evaluation [53, 54]. Tissue biopsy is warranted before cancer treatment when malignancy is suspected [55]: SVCS often develops gradually and may allow treatment delay for definitive diagnosis [4, 46].

Management. Nursing management includes bed rest with a Fowler or semi-Fowler position to elevate the patient's head to facilitate venous drainage and oxygen therapy. Systemic corticosteroids (and diuretics) are frequently used (particularly during radiotherapy), even if their efficacy is largely unproven except in lymphomas [46, 55]. Therapeutic choice may be different according to the clinical severity of SVCS and underlying disease. For highly chemo-responsive disease such as lymphoma, germ cell cancer, small cell lung

cancer [4, 46], and possibly BC [56], CHT may be the preferred treatment. Targeted therapy may be indicated according to pathological evaluation. Radiotherapy induces a rapid symptomatic improvement in 74–95% of patients within 3–14 days [57] and may be part of multimodal treatment in metastatic BC. Intravascular stent placing is reserved to cases with severe clinical presentation and limited efficacy or recurrence after medical treatment [46]. Heparin is generally administered around the procedure, but the need for long-term anticoagulation remains controversial [52]. Complications of stent placing are rare, but possibly severe with 2% of mortality rate [58]. Surgery, although effective, requires careful patient selection in view of morbidity and 5% mortality rate [59].

58.3 Metabolic Emergencies

58.3.1 Hypercalcemia

Hypercalcemia of malignancy (HCM) is the most frequent cause of hypercalcemia in the hospital setting [60–62]. It affects 3–30% of cancer patients, particularly with advanced disease. Improved recording may explain increasing prevalence [62], while the widespread adoption of bisphosphonates (BPs) may limit its occurrence [61]. Lung cancer, BC, and multiple myeloma (MM) are the most frequent causes of HCM, followed by cancers of the head and neck, kidney, and ovary [63].

Pathophysiology. The total serum calcium, normally ranging from 8.9 to 10.3 mg/dL, is the sum of the free ionized calcium (47%), the sole component with biological activity, and the non-ionized calcium, mainly bound to albumin and to a lesser extent to serum anions [63]. Accordingly, calcium values should be more conveniently reported as a function of the patient's albumin or "corrected serum calcium" [61, 63].

Four types of HCM have been identified [61]. The most frequent is humoral HCM (about 80% of cases), often with minimal or absent bone involvement and caused by secretion of parathyroid hormone (PTH)-related protein (PTHrP) by malignant tumors (mainly squamous cell cancer, renal cancer, ovarian cancer, endometrial cancer, HTLV-associated lymphoma, BC). The second most common type of HCM, "local osteolytic hypercalcemia" (about 20% of all cases, mainly BC, MM, or lymphoma), affects patients with high bone metastatic tumor burden. Less frequent forms of HCM may be related to excess vitamin D activation (absorptive hypercalcemia) or to ectopic secretion of PTH [61, 63].

The PTHrP protein binds to the same PTH receptor (PTH1R) and stimulates bone resorption with calcium and phosphate release through increased osteoblast receptor activator of nuclear factor kB (RANK) ligand expression, leading to activation of RANK located on osteoclast precursors. It is worth remembering flare hypercalcemia in metastatic BC treated with tamoxifen [64, 65] and less frequently described after treatment with aromatase inhibitors [66–68].

Clinical Features. Clinical manifestations, not precisely related to the severity of hypercalcemia but also to the rate of calcium increase and to albumin level, may be initially nonspecific with possible diagnosis delay [61]. Classic symptoms comprise gastrointestinal manifestation with anorexia, nausea, vomiting, weight loss, abdominal pain, constipation, pancreatitis [63], bone pain revealing skeletal disease involvement, polyuria, fatigue, and weakness. Higher calcium level can lead to renal failure, progressive neurological impairment culminating in coma, and death. Patients with HCM may have short survival, but poor prognosis could reflect advanced cancer stage [63]. Median survival after diagnosis of HCM in BC was 4.5 months and decreased to only 3 months after exclusion of patients with flare HCM. The interval between the first relapse and HCM, sites of metastases, primary treatment, and the level of serum alkaline phosphatase were independent prognostic factors. In a palliative setting, patients not receiving anticancer treatment calcium response to BPs had a significantly positive effect on survival [69].

Diagnosis. The evidence of advanced cancer disease at the time of clinical onset often suggests diagnosis. Laboratory investigation includes measurement of calcium, of albumin, and of intact PTH which should be suppressed. Serum and urine electrophoresis with serum and urine immunofixation are indicated if MM is suspected.

Management. Fluid rehydration is almost universally prescribed. Diuretics, though used, have no proven benefit. Intravenous BPs are treatment of choice, but patients may have incomplete response in 21% or relapse after treatment in 24% of cases [70]. Denosumab may be better than zoledronic acid in preventing skeletal-related events or HCM in advanced malignancies involving bone [71] with 52% lower incidence of HCM in BC [72, 73]. Intensive denosumab schedules may allow calcium control in patients with BP-refractory HCM; however, adverse events were reported [74] and caution was suggested, particularly in patients with renal dysfunction, for the risk of fatal hypocalcemic episodes [75].

58.4 Hyponatremia and SIADH

Hyponatremia (HN) is the most common electrolyte disorder in patients with cancer and possibly associated with decreased survival. Approximately 14% of HN in medical inpatients is related to cancer [76, 77].

Pathophysiology. Hyponatremia is defined as a serum sodium level <135 mmol/L [78]. In most cases, HN in cancer

is induced by syndrome of inappropriate antidiuretic hormone (SIADH), firstly described by Schwartz in 1957 [79], where antidiuretic hormone (ADH) increased release by the pituitary gland or from ectopic production occurs independently from effective serum osmolality or circulating volume [80]. Cancer types most frequently associated with SIADH are small cell lung cancer (10–15%) and head and neck cancer (3%), but it has been identified in a wide variety of other solid and hematological malignancies, including BC [80].

Antineoplastic drugs, especially vincristine, vinblastine, cyclophosphamide, and cisplatin, may cause SIADH [77, 81].

Clinical Features. Hyponatremia can be asymptomatic, or neurological symptoms can appear related to brain edema and increased intracranial pressure.

Milder symptoms, more frequent in patients with chronic hyponatremia, are nausea, headache, confusion, attention deficit, gait disturbances, and risk for falls. Large or rapid declines in serum sodium may cause seizures, coma, and cardiorespiratory arrest [77, 78].

Diagnosis. Evaluation of plasma osmolality is the first step for differential diagnosis. It will be within the normal range in pseudo-HN, a laboratory artifact that is related to high blood concentrations of lipids or proteins interfering with measurement of sodium.

A high plasma osmolality (> 280 mOsm/kg, hypertonic HN) can be due to the presence of osmotically active substances, such as glucose or mannitol, inducing HN by dilution.

In most cases of hyponatremia, plasma osmolality will be low (<280 mOsm/kg, hypotonic HN). In these cases, the next step is to assess urine osmolality. If urine osmolality is <100 mOsm/kg, renal diluting mechanism is intact, and this suggests excessive water intake as a cause of HN. If urine osmolality is >100 mOsm/kg, an inappropriate renal dilution and impaired water excretion are likely.

Evaluation of volume status of the patient can help to distinguish between:

- Hypovolemic hyponatremia: decrease in both total body water volume and sodium, but with the decrease in sodium exceeds the water volume, a condition due to renal sodium loss (e.g., use of thiazide diuretics, gastrointestinal loss).
- Euvolemic hyponatremia: increase in total body water while sodium is near normal, a condition due to SIADH with water retention and urine sodium elimination.
- Hypervolemic hyponatremia: increase in both the total body water volume and sodium, but the increase of water volume exceeds sodium, a condition due to inappropriately elevated amounts of ADH (renal failure, heart failure, cirrhosis). The patient has volume overload or edema. [77, 82, 83].

Management. Medications that may cause hyponatremia should be discontinued.

Hypovolemic patients should be treated with 0.9% saline.

Hypervolemic patients need therapy of the underlying pathology, but usually can be treated safely with loop diuretics.

For euvolemic, mild, or moderate HN that is chronic and minimally symptomatic, there is consensus against a treatment. Fluid restriction is the cornerstone of therapy. In case of acute, symptomatic, moderate, or profound HN, 3% of hypertonic saline is indicated to gradually correct the HN. Correction of sodium should not be too rapid for the risk of neurological "sequelae" due to osmotic demyelination.

If a diagnosis of SIADH is made and HN is refractory to fluid restriction and saline infusion, guidelines recommend increasing intake of osmotic solutes (oral urea). Demeclocycline and vasopressin receptor antagonists (vaptan drugs) can increase serum sodium, but they have limited clinical experience and concerns on safety [78–80, 84].

58.4.1 Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) is a cancer complication generated by tumor cell damage and release into the bloodstream of cellular components with complex metabolic and electrolytic perturbation [4]. It is a common complication in patients with hematological malignancies and less frequent in solid tumors [85]. Sporadic cases have been reported also in BC, sometimes after single doses of CHT [86–88].

Pathophysiology. Tumor cell lysis causes rapid release in the bloodstream of intracellular potassium, phosphate, and nucleic acids (purines). Purines are catabolized to hypoxanthine, then to xanthine, and finally to uric acid by the enzyme xanthine oxidase. Uric acid (UA) can crystalize and obstruct the flow in the renal tubules [89, 90]; plasma phosphate and calcium phosphate crystalize in soft tissue, including the renal tract. Renal injury causes hyperkaliemia and acidosis, thus promoting further UA crystallization.

Clinical Features. Cairo classification [89] distinguishes a clinically silent, laboratory-detected TLS and a TLS with clinical manifestations.

Clinical manifestations may include nausea, vomiting, lethargy, edema, fluid overload, congestive heart failure, cardiac dysrhythmias, seizures, muscle cramps, tetany, syncope, and possibly sudden death. The clinical onset may start prior to, or more commonly within 12–72 h after, administration of cytotoxic therapy.

A grading of TLS severity on the basis of clinical complications is also defined [90].

Diagnosis. Diagnostic criteria for TLS have been indicated by Cairo-Bishop in 2004. Laboratory TLS is defined as the presence of two or more of the following abnormalities in a cancer patient within 3 days prior to and up to 7 days after initiation of treatment: plasma levels of UA ≥8 mg/dL,

potassium ≥6 mEq/L, phosphorus ≥4,5 mg/dL, and/or calcium ≤7 mg/dL. Clinical TLS is diagnosed in a patient with laboratory TLS and at least one of these: creatinine serum level ≥1.5 ULN, cardiac arrhythmia, seizure, and sudden death [89].

High morbidity and mortality associated to TLS require identification of patients at risk [90]. Patient-related predictors are older age and low glomerular filtration rate, volume depletion, nephrotoxic medications, and high baseline serum UA, phosphorus, potassium, and lactate dehydrogenase. Tumor-related risk factors include type and burden of disease, high proliferation rate, and high sensitivity to anticancer therapy [86, 91]. These factors allow for patients stratification into three main risk groups [92].

Management. Prevention of TLS is the appropriate strategy. Hydration with intravenous fluid infusion is recommended in intermediate- and high-risk patients to ensure urine output of at least 2 mL/kg/h to minimize risk of kidney injury, before initiation of CHT [90, 93]. Prophylactic oral xanthine oxidase inhibitor, allopurinol, which is highly effective to reduce the conversion of xanthine and hypoxanthine to UA, should be used at the dose of 200–400 mg/m^2/day in one to three doses, up to a maximum of 800 mg a day, given for up to 7 days after CHT is started. Allopurinol's therapeutic effect is delayed by 24–72 h [90], while rasburicase, a recombinant form of the enzyme urate oxidase, reduces plasma UA metabolizing it to allantoin, a much more soluble product. In patients with high risk of developing TLS, rasburicase should be offered along with hydration, as a single dose of 0.1–0.2 mg/kg or as a fixed dose of 3 mg, and repeated only if clinically necessary [90, 92]. Urinary alkalinization increases uric acid solubility but decreases calcium phosphate solubility and should be avoided if rasburicase is available [94].

Once TLS has developed, frequent clinical monitoring and intensive care physicians are indicated. The first step is to maintain a high urine output with hydration with careful fluid balance monitoring. The use of diuretics is controversial because hypovolemia may further compromise kidney function. Rasburicase is the drug of choice for TLS at the dose of 0.2 mg/kg daily, continued for 3–7 days, on the basis of clinical response. Correction of hyperkalemia and hypocalcemia may be needed. Hemodialysis should be considered in patients who are anuric with fluid overload and who have refractory hyperkalemia, hyperuricemia, hyperphosphoremia, and/or symptomatic hypocalcemia [90, 91].

58.5 Hematologic Emergencies

58.5.1 Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is a rare hemostatic disorder characterized by activation of clotting and fibrinolytic systems and possible occurrence of bleeding and/or thrombosis. It may be associated with cancer or other diseases, sometimes as the first clinical manifestation [95, 96].

It is reported in approximately 15% of patients with acute leukemia [97] and 6.8% of patients with solid tumors [98] with older age, male gender, advanced malignancies, BC, and tumor necrosis being risk factors. Acute DIC was reported in 1.1% of patients with advanced BC [99].

Pathophysiology. Cancer-related DIC can be due to cancer itself, cancer treatment, or infections [100]. Activation of the blood coagulation pathways is triggered by tissue factor generated by the tumor cells or by host monocytes-macrophages with excessive production of thrombin within the intravascular space. An increased expression of tissue factor can also be observed on endothelial cells, strictly involved in the regulation of blood coagulation by protein C activation, the tissue factor pathway inhibition, or thrombomodulin, and in the same way pro-inflammatory cytokines or tumor necrosis factor can trigger procoagulant pathways, even exacerbating a possible role of CHT or any other treatment [101].

The consumption of platelets and hemostatic factors, the deposition of fibrin in the microcirculation, and the activation of the fibrinolytic system are the main following pathogenetic events and often result in an increased risk of bleeding. Vascular fibrin deposition may cause micro- or macrothrombosis, organ dysfunction, or microangiopathic hemolytic anemia [100].

Clinical Features. The clinical expression of DIC in cancer patients can be widely variable. A clinical distinction can be made between thrombotic, hemorrhagic, or silent forms suggested by laboratory tests. Likewise acute, subacute, or silent forms can be distinguished [101]. Limited data are available about clinical presentation of DIC in BC; although neither acute nor thrombotic/hemorrhagic forms can be excluded, subacute or silent forms seem a more frequent expression in BC (Table 58.2).

Diagnosis. Evidence of hemorrhage and/or thrombotic manifestations together with laboratory findings, including thrombocytopenia, prolonged prothrombin time or activated partial thromboplastin time, decreased serum fibrinogen levels, elevated D-dimer levels, and microangiopathic anemia with schistocytes in the peripheral blood smear, may suggest DIC [102]. Bone marrow metastases can be present [96, 103]. A diagnostic algorithm and score system are useful to assess the probability of DIC (Table 58.3) [101].

Treatment. Treatment of the underlying cause is the primary objective for an effective control of any form of DIC. As most patients have a disseminated cancer at the time of DIC presentation, systemic treatment is the only possible therapy. The choice of an appropriate CHT regimen can be difficult. The risk of serious myelosuppression, also in view

Table 58.2 Clinical manifestations and clinical approach in cancer-related DIC

	Procoagulant	Hyperfibrinolytic	Subclinical
Predominant kinds of cancer	Pancreatic cancer, adenocarcinoma	Acute promyelocytic leukemia, prostate cancer	The most part of solid tumors (breast cancer)
Predominant clinical symptoms	Thrombosis	Bleeding	No symptoms
Clinical presentations	Signs or symptoms of arterial ischemia, cerebrovascular manifestations, venous thrombosis or pulmonary embolism, noninfectious endocarditis	Abnormal hemorrhagic symptoms	Only laboratory abnormalities, without any sign or symptoms of thrombotic or hemorrhagic syndrome
Treatment	Underlying cancer Anticoagulation with heparin	Underlying cancer Supportive care with blood products	Underlying cancer Anticoagulation with heparin

Table 58.3 ISTH score for DIC diagnosis [101]

Laboratory result	Score
Platelet count	
>100,000/mm^3	0
50,000–100,000/mm^3	1
<50,000/mm^3	2
Fibrinogen level	
>100 mg/dL	0
<100 mg/dL	1
Prothrombin time (PT)	
Prolonged <3 s	0
Prolonged 3–5 s	1
Prolonged ≥6 s	2
D-Dimer or fibrin degradation products	
No increase	0
Moderate increase	2
High increase	3
Diagnosis of DIC can be made when a score of ≥ 5 is associated with clinical signs or symptoms of thrombotic and/or hemorrhagic syndrome	

of the frequent bone marrow involvement and preexisting thrombocytopenia, can precipitate bleeding; patient often has large tumor burden with liver involvement and reduced performance status. Interestingly a weekly CHT regimen with high dose of 5-fluorouracil and leucovorin infused over 24 h showed low marrow toxicity and efficacy in patients with gastric cancer and DIC [104] and safety in patients with organ dysfunction [105]. Unfortunately it was not very successful in patients with BC and DIC, even if efficacy was apparently improved when vinorelbine was added [99].

Supportive care may be important even if it can place the dilemma of balancing between the risk of thrombotic and hemorrhagic manifestations.

Prophylactic heparin anticoagulation may be suggested in patients with cancer-related DIC, with the exception of hyperfibrinolytic DIC or other contraindications. Heparin is indicated in patients with predominating thrombotic manifestations with regular clinical and laboratory surveillance [106]. Recombinant human soluble thrombomodulin (TM-α) could become a possible alternative to heparin [107].

In patients with active bleeding, platelet transfusion to maintain the platelet count above 50,000/mm^3 is suggested. Patients at high risk of bleeding (e.g., surgery or invasive procedures) should receive prophylactic transfusions when platelet count is less than 20,000/mm^3.

Fresh frozen plasma (15–30 mL/kg) is suggested in case of active bleeding. Prothrombin complex concentrates can be preferred in case of concerns over volume overload. When low fibrinogen values (below 1.5 g/L) persist despite these supportive measures, transfusion of cryoprecipitate (whenever available) or fibrinogen concentrate is an option [106].

58.6 Treatment Related

58.6.1 Febrile Neutropenia (FN)

Neutropenia is a common effect of anticancer CHT and often goes asymptomatic until hematological recovery; however, infection and febrile neutropenia (FN) may arise, with possible complications, hospitalization, increased costs, treatment delays or discontinuations, dose reductions, and death [108, 109].

The Infectious Diseases Society of America defines FN as a disorder characterized by an absolute neutrophil count (ANC) <1000/mm^3 and a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥38 °C (100.4 °F) for more than 1 h [110]; other definitions are quite similar [110–117]. In a recent survey, FN occurred in 15.1–21.5% of patients undergoing CHT for metastatic BC, with a 2.1–6.2% rate after the first cycle [110].

Pathophysiology. Incidence and severity of infection are inversely proportional to the ANC, with increasing risk as the ANC falls below 500/mmc and especially under 100/mmc; however, also the ANC fall rate, neutropenia duration, and other predisposing factors are important. The damages of mucosal membranes induced by CHT, any interruption to the integument integrity (e.g., venipuncture and indwelling vascular catheter), the damage of the ciliary function of the trachea and bronchi, any form of obstructive

phenomenon, and alteration of microbial flora may allow bacteria and/or fungi, already colonizing the patient, to permeate the host tissues through his/her mucosal barriers [108]. Neutropenic fever syndromes may fall in three categories: microbiologically documented infection, clinical documented infection, and unexplained fever. Common sites of infections are the oropharynges, the lungs, the perianal area, and the skin.

The most frequently isolated microorganisms from blood culture in patients with FN are Gram-positive cocci, including *Staphylococcus aureus*, *Staphylococcus epidermidis* (especially in patients with indwelling devices), *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Viridans streptococci*, and *Enterococcus faecalis* and *Enterococcus faecium*. Gram-negative bacilli include *Escherichia coli*, *Klebsiella* species, and *Pseudomonas aeruginosa*. *Candida* is the most common fungal infection [118].

Clinical Features. As inflammatory response may be decreased in neutropenic patients, cutaneous, soft tissue, and pulmonary infections may not be obviously clinically or radiologically recognizable; fever can be the sole sign of an initially localized infection [108]. Glucocorticoids may be a confounding factor, limiting the febrile response to bacteria on pyrogens [119]. Accordingly attention should be paid in case of tachycardia, tachypnea, or hypotension in afebrile neutropenic patients as signs of incipient septic shock.

Diagnosis. The initial evaluation should include [110–113]:

- A detailed history with particular attention to new site-specific symptoms, antimicrobial prophylaxis, infection exposures, prior documented infections or pathogen colonization, and underlying comorbid disease.
- A careful clinical examination of the patients with cardiovascular and respiratory assessment and evaluation of the skin, oropharynx, perineum, maxillary and frontal sinuses, abdomen, as well as vascular access site.
- Laboratory tests comprising a complete blood count and measurement of serum levels of creatinine and blood urea nitrogen, electrolytes, liver enzymes, and bilirubin and blood cultures. Chest radiograph, particularly for patients with respiratory signs or symptoms, should be considered.
- Assessment of risk factors for complications of FN [112]. A validated tool is the Multinational Association for Supportive Care in Cancer (MASCC), a simple scoring system with a maximum theoretical score of 26. Patients with a score < 21 are considered as high risk (Table 58.4) [120].

Table 58.4 MASCC scoring index [120]

Characteristic	Score
Burden of illness: no or mild symptoms	5
Burden of illness: moderate symptoms	3
Burden of illness: severe symptoms	0
No hypotension (systolic BP >90 mmHg)	5
No chronic obstructive pulmonary disease	4
Solid tumor/lymphoma with no previous fungal infection	4
No dehydration	3
Outpatient status (at onset of fever)	3
Age <60 years	2

Management. Therapy of FN must be prompt, empiric, bactericidal, and broad spectrum [108]. High-risk patients require hospitalization and IV empirical antibiotic monotherapy with an antipseudomonal beta-lactam agent, such as piperacillin-tazobactam, cefepime, or carbapenem. Vancomycin should be added only in case of suspected catheter-related infection, skin or soft tissue infection, pneumonia, or hemodynamic instability. Modification of treatment should be considered on the basis of clinical and microbiologic information [110].

Low-risk, hemodynamically stable patients may receive oral and/or outpatient treatment, with ciprofloxacin plus amoxicillin-clavulanate, levofloxacin, or ciprofloxacin monotherapy, with hospital admission in case of worsening conditions [121]. For high-risk patients, with persistent fever after 4–7 days of antibiotic treatment without identified fever source, empirical antifungal treatment should be considered. Antibiotic treatment should be continued at least until ANC recovery of ≥500/mmc in patients being afebrile for 48 h with negative blood cultures.

Prophylactic treatment with fluoroquinolone and antifungal agents is generally considered for high-risk patients with expected, prolonged, and profound neutropenia (ANC to ≤100/mmc for ≥7 days).

Routine use of granulocyte colony-stimulating factors (G-CSF), for treatment of uncomplicated FN, is not recommended, except for patients at higher risk of complications, not responding to adequate antibiotic treatment, and with life-threatening infectious complications [113–116].

Primary G-CSF prophylaxis is recommended when FN risk exceeds a threshold of 20% [113, 115, 122]. When FN risk is 10–20%, patient-related risk factors, such as age ≥65 and comorbidities, should guide the decision [113–116]. Secondary prophylaxis with CSFs is indicated after a previous FN episode. An expanded list of CHT regimens at high (≥20%) or intermediate (10–20%) risk of FN is available [113, 116]. However, it must be remembered that FN rates could be higher in the real-world nonselected patients [118]. Several FN predictive tools have been developed, even if few have been prospectively validated [120, 123, 124]. Finally education of outpatients with detailed and clear instruction regarding symptoms and referral contacts should be provided [111].

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59 Breast Cancer (Diagnosed) During Pregnancy: Adapting Recent Advances in Breast Cancer Care for Pregnant Patients

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59.1 Introduction

Breast cancer diagnosed during pregnancy (BCP) is rare, but an increased awareness of treatment options has led to more intensive breast cancer treatment during pregnancy in recent years. The recommendations for diagnosis and treatment of BCP, first published in 2006 and updated in 2010, aimed to increase awareness that treatment during pregnancy is the first option [1, 2], adhering as closely as possible to the general recommendations for young non-pregnant women.

This article aims to provide guidance on BCP with a focus on novel data on BCP and recent advances in breast cancer therapy, including the use of carboplatin, dose-dense chemotherapy, trastuzumab, neoadjuvant therapy and sentinel lymph node biopsy (SLNB) as sole treatment, and how these can be adapted to the needs of pregnant patients (Table 59.1).

59.2 Methods

Members of the breast cancer guideline consortium of the German Cancer Society (DKG) (AGO Kommission Mamma; SL, GvM, CT); the International Network on Cancer, Infertility and Pregnancy (INCIP) of the European Society of Gynaecological Oncology (ESGO; FA, SL, PB, KVC); and other internationally renowned experts in the fields of breast cancer and placenta research (AP, BK, CK, CD, HS, AS, OG, PP, UM) reviewed the literature on BCP, with a focus on 'when the general recommendation to treat as closely as

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Table 59.1 Updated recommendations for breast cancer diagnosis and treatment in pregnant and non-pregnant women incorporating recent advances

	Non-pregnant women	Remarks for pregnant women
Diagnostic		
Ultrasound		The preferred technique
Mammography	Techniques with lower exposure	Bilateral mammography recommended in case of BC
MRI and PET	Not generally recommended	Not recommended during pregnancy
Targeted treatment		
Endocrine treatment	GnRH + aromatase inhibitors or tamoxifen	Not indicated
Trastuzumab and pertuzumab	Pertuzumab in addition to trastuzumab for neoadjuvant-treated patients	Risk/benefit analysis needs to be discussed, as early start of trastuzumab improves survival. However, foetal toxicity and oligo-/anhydramnios need to be considered. No data for pertuzumab
Chemotherapy		
Anthracyclines		Transplacental transport, while low, is higher vs taxanes. PK unchanged vs non-pregnant women
Taxanes	Paclitaxel and docetaxel are used mainly in sequential regimen. Weekly paclitaxel is the preferred taxane	Transplacental transport is very low. Small series PK seems to be lower in pregnant vs non-pregnant women, *but dose according to actual body weight and use dose for non-pregnant women*. *Prefer paclitaxel to docetaxel*
Nab-paclitaxel	Higher pCR rate in one study vs. paclitaxel but no long-term data	No data during pregnancy, not indicated
Carboplatin	May be considered for neoadjuvant therapy in TNBC ± g*BRCA* mutation carriers	May be considered for neoadjuvant therapy in TNBC ± g*BRCA* mutation carriers
5-FU	Does not demonstrate added value in non-pregnant women	5-FU-containing regimen not indicated during pregnancy
Preferred regimen		
Standard *EC/AC q3w/Pac q1w*	Taxane based: EC every 3 weeks followed by paclitaxel weekly is one of the most widely used regimens; long-term follow-up recently confirmed activity (reverse sequence is possible)	Taxane based: EC every 3 weeks followed by paclitaxel weekly (reverse sequence is possible—decision might be based on gestational age)
EC/AC q3w/Doc q3w	An almost equally effective regimen—higher myelotoxicity, less sensory neuropathy	An option decision based on side effects and experience
DAC	As effective as AC-Doc, less frequently used because of higher toxicity	Not recommended during pregnancy, because better evaluated and less toxic regimen available
Dose-dense regimen *EC/AC q2w/Pac q1w*	One of the standard[a/b]regimen and an alternative to *EC/AC q3w/Pac q1w*	Can be considered as an option in higher risk BCP patients –G-CSF obligatory
EC/AC q3w/Pac q2w	See above[a]	No data in BCP
ACPac/AC-Pac q2w	See above[a/b]	AC q2w/Pac q2w seems to be an alternative in BCP patients [3]
Dose-dense and intensified dose-dense CT E-Pac-C q2w	Dose-dense and intensified dose-dense CT can be considered in certain high-risk patients	Intensified dose-dense CT is not recommended: high risk for febrile neutropenia and anaemia with need for transfusion
Surgery		
BCS/mastectomy		Indication as in non-pregnant women
Sentinel lymph node biopsy	SNLB is one standard procedure for a certain group of women It is a standard diagnostic procedure for cN0 women	Higher evidence to support use during pregnancy—use to be discussed in pregnant women. Radioactive tracer preferred. Use adapted 1-day protocol

Table 59.1 (continued)

	Non-pregnant women	Remarks for pregnant women
Immediate breast reconstruction		One series during pregnancy reported insertion of an expander as an option. Further, breast reconstruction, e.g. a flap, is not a standard option during pregnancy—breast size differs between pregnant and non-pregnant status

BC breast cancer, *MRI* magnetic resonance imaging, *PET* positron emission tomography, *GnRH* gonadotropin-releasing hormone, *CT* chemotherapy, *PK* pharmacokinetics, *EC* epirubicin/cyclophosphamide, *pCR* pathological complete response, g*BRCA* germ line BRCA, *TNBC* triple-negative breast cancer, *5-FU* 5-fluorouracil, *BCS* breast-conserving surgery, *SLNB* sentinel lymph node biopsy, *cN0* baseline node negative
[a]According to www.ago-online.de; Commission Mamma Version 14.1.0 (March 2015)
[b]According to NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. Version 2.2015

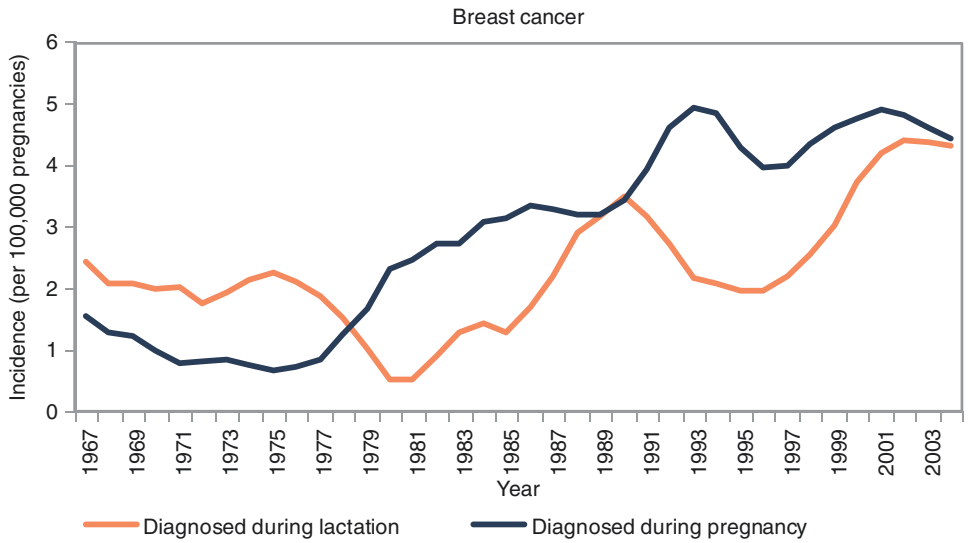

Fig. 59.1 Incidence of breast cancer during pregnancy and lactation (up to 6 months after delivery) based on data extracted from the Cancer Registry and the Medical Birth Registry of Norway. The figure shows annual incidence of BC during pregnancy, *blue line*, for the period 1967–2004, using 5 years of moving averages, showed as proportions per year per 100,000 pregnancies. The mean annual incidence during lactation is about 1.7/100,000 and during pregnancy 3.3/100,000. For the period 1990–2004, the incidence of BC during pregnancy was 4.6/100,000, which is about 1/20,000. For the pregnancy-associated breast cancer (PABC) period, about 1% of all pregnancies are affected. The data are extracted from the material used in the publication by Stensheim et al. [5]

possible to non-pregnant women cannot be followed'. The aim was to provide a narrative review on this special patient cohort providing an update on current understanding of the disease, as well as diagnostic and treatment considerations. A meeting under the umbrella of the DKG and the Union Internationale Contre le Cancer (UICC), supported by DKG and the German Breast Group (GBG) Foundation, was held to discuss the draft recommendations.

59.3 Epidemiology

Breast cancer is one of the most common malignancies during pregnancy [4, 5]. Overall, the incidence of BCP has been increasing during the last few decades (Fig. 59.1). Maternal age has been on the rise in developed countries since the 1970s and subsequently also in several developing countries [6, 7]. The upward trend of breast cancer incidence and the postponing of childbearing have increased the numbers of BCP cases [4, 5, 8, 9]. Approximately one in five breast cancers diagnosed in the age group 25–29 years are associated with a pregnancy, either diagnosed during pregnancy or during the first postpartum year. The reported occurrence of breast cancer diagnosed during pregnancy ranges from 2.4 to 7.3 per 100,000 pregnancies in population-based investigations [4, 5, 8, 9].

A Danish study found that 81% of pregnancies affected with breast cancer were terminated during the first trimester, while others report that only 19% of all BCP was diagnosed in the first trimester [9, 10]. Delayed diagnosis is a likely explanation for the lower number of observed versus expected breast cancer cases.

59.4 Prognosis

Breast cancer during pregnancy generally presents in more advanced stages when compared to that in non-pregnant women, potentially resulting in an overall worse outcome [11]. Several previous studies have addressed this issue, but due to small numbers, results were inconsistent [12, 13]. The largest cohort study included 313 patients controlling for stage, prognostic factors and adjuvant treatment; survival was similar for patients with BCP versus non-pregnant breast cancer patients [14]. In contrast to breast cancer diagnosed during the first year after delivery, the diagnosis of breast cancer during pregnancy does not seem to be an independent poor prognostic factor, provided that standard treatment is administered. Despite possible pharmacokinetic changes, survival rates did not differ between patients who received chemotherapy during pregnancy versus after delivery [10].

59.5 Diagnosis

59.5.1 Imaging Diagnostics

The general recommendations have not changed since 2010. Breast imaging and staging require separate consideration. Breast ultrasound and mammography can be safely and effectively performed during pregnancy [15, 16]. Bilateral mammography is recommended in all women with a confirmed or highly suspicious malignant lesion. The radiation dose is less than 3 mGy, which corresponds to approximately 7 weeks of background radiation [17]. The estimated dose to the uterus/foetus is less than 0.03 μGy [18]. Nevertheless, many patients and physicians are concerned about radiation safety, and this should be discussed with the patient. 'In general, maternal and fetal radiation exposure and dose are affected by gestational age, anatomic site, modality, and technique [16]'. The threshold for negative effects of radiation on the foetus is around 100 mGy, with uncertainty at doses between 50 and 100 mGy [19]. Contrast-enhanced MRI is not recommended during pregnancy. The use of iodinated and gadolinium-based contrast agents during pregnancy is insufficiently explored. The imaging/staging procedures should be conducted only in advanced stages where they might alter the treatment. Unnecessary and less accurate staging procedures should be avoided, as in non-pregnant breast cancer patients. Whole-body MRI has not been studied sufficiently in breast cancer in general. Although pharmacologic agents used for diagnostic nuclear medicine and PET probably do not result in radiation exposure exceeding 50 mGy, they are not recommended during pregnancy [20]. All palpable masses require imaging and imaging-guided biopsy without delay.

59.5.2 Pathology

Histopathologic diagnosis based on core biopsy of the suspicious lesion is the gold standard for BCP. The pathologist needs to be informed about the pregnancy. Overall, the histological features of BCP tumours do not differ from those in young non-pregnant women with breast cancer [10]. The vast majority are ductal invasive, mainly hormone-receptor negative and undifferentiated. In general, tumour mutations do not differ between pregnant and non-pregnant young women, although small series showed significant differences in gene expression analyses [21]. No definite conclusions for general practice can be drawn so far from these analyses. The main challenge for future research is the selection of an appropriate control cohort. Matching cohorts by treatment and/or histology, as well as by age, is required.

59.5.3 BRCA Testing

Family history-taking is a prerequisite, and genetic counselling should be offered according to national guidelines, which differ significantly between countries. BRCA testing will become treatment relevant. The majority of BCP are triple-negative (TNBC); in young TNBC patients, the probability of detecting a germ line *BRCA* mutation is around 20% [22].

59.6 Local Treatment

59.6.1 Surgical Treatment

In general the surgical approach is the same as for non-pregnant patients. Mastectomy is not recommended solely on the basis of pregnancy and possible consequent delay of the radiotherapy. The general recommendations are detailed in a recent publication [23]. Immediate breast reconstruction after mastectomy is an essential component in managing breast cancer patients, particularly those diagnosed at a young age. Based on a single published experience, tissue expander insertion appears to ensure a short operation time and does not seem to be associated with considerable morbidity to the patient or the foetus. Hence, this surgical technique could be considered in the multidisciplinary management of women diagnosed with breast cancer during pregnancy [24].

59.6.2 SLNB

Recommendations from ASCO still state that pregnant patients should not undergo SLNB, based on cohort studies and/or informal consensus [25]. However, it has been shown

that this procedure can be safely performed during pregnancy [26, 27]. SLNB involves locoregional administration of relatively low injected radioactivity doses, with rapid clearance of the negligible radioactivity in the body, as well as substantial and stable uptake at the injection site—which is shortly thereafter removed by surgery. Considering the radiopharmaceuticals and the amounts of activity typically used for SLNB in optimised protocols, the doses absorbed by the foetus are mostly below 20 μGy for 10–20 MBq (about 1 μGy/MBq), as assessed by experimental results and Medical Internal Radiation Dose (MIRD) Committee models [28, 29].

From a maternal oncologic point of view, SLNB appears to be accurate and safe, with only one unsuccessful mapping and one recurrence among 97 patients with BCP [30, 31]. Pregnant breast cancer patients should be offered SLNB rather than axillary clearance whenever it is indicated according to general practice in non-pregnant patients. It is advisable to inject colloid in the morning (1-day protocol) in order to minimise radiation exposure. Blue dye as a sole procedure is not recommended outside pregnancy and is therefore not an option in BCP, because of the low (1%) but potentially harmful underlying risk of an anaphylactic maternal reaction [32, 33]. In a small series of 25 women with SLNB during pregnancy, 7 received blue dye for mapping [31].

59.6.3 Radiation Therapy (RT)

RT during pregnancy is rarely indicated in BCP. In general it is recommended to postpone RT until after delivery [34]. The available information on long-term consequences of in utero exposure to RT is limited [35]. The two factors that have to be considered when RT during pregnancy is indicated are the dose to the foetus and the risk that radiation causes side effects to the foetus. It is important to relate the latter to the magnitude of spontaneously occurring abnormalities. Deterministic (teratogenic) effects must be discriminated from stochastic (carcinogenic) effects. The former are dose dependent and occur only above a certain threshold, while the severity of the latter is independent of the dose, although the probability is dose dependent and without a threshold.

In early pregnancy (when it may not have been diagnosed), irradiation will generally lead to spontaneous abortion, while from the third week onwards, malformations can occur. Radiation may influence the development of the central nervous system, possibly inducing neuropsychological and behavioural dysfunction. The main stochastic effect is the induction of childhood cancer and leukaemia. At low doses the incidence of childhood cancer and leukaemia (0.2–0.3% for ages 0–15 years) does not seem to be increased. Following a dose of 10 mGy, the relative risk increases to 1.4, still resulting in a low absolute excess risk [36]. Another stochastic effect is the induction of germ line mutations to the oocytes; however, there is no evidence of negative effects in humans (Table 59.2).

Table 59.2 Overview of risks and threshold doses following radiation to the foetus

Stage of pregnancy (weeks)	Risk	Threshold dose
<2	Spontaneous abortion	None
3–8	Malformations	100–200 mGy
8–25	Disturbed CNS development	50 mGy
0–40	Childhood cancer/ leukaemia	None

The radiation dose received by the foetus depends on the distance between the RT field and the position of the foetus, so is dependent on gestational age, as well as the amount of leakage of irradiation outside the radiation field and the use of effective shielding, which can reduce the dose by 50–75%. During the first months of pregnancy, the uterus does not extend outside the true pelvis, and, provided that appropriate techniques and shielding are used, the dose to the foetus will be only 0.1–0.3% of the prescribed dose to the breast, resulting in a very low risk of inducing malformations [35]. Several cases with RT administered for BCP are reported, with low foetal doses and resulting in the delivery of healthy babies [37]. Therefore, RT might be considered in the first or early second trimester, if the risk of delaying or omitting RT is felt to outweigh that of harming the foetus.

59.7 Systemic Therapy

59.7.1 Chemotherapy

Chemotherapy is contraindicated during the first trimester of pregnancy because of a higher risk of inducing foetal malformations. The (US) National Toxicology Program (NTP) monograph reports a prevalence of malformations of 14% if chemotherapy is given in the first trimester, declining to 3% if chemotherapy is applied later in gestation [38]. In comparison, the reported rate of major malformations in the general population is approximately 3% in the US and 6.7% in a German registry [39, 40]. Postponing chemotherapy treatment until after delivery might seem to be an option. However, data in non-pregnant young women indicate that delaying/postponing chemotherapy might increase the risk of relapse [41]. Therefore, it is recommended to treat women with BCP during the second and third trimester, following guidelines for non-pregnant young patients as closely as possible [1, 2]. Some anticancer agents, such as trastuzumab, tamoxifen and endocrine agents, should in general be avoided during pregnancy, given their potential foetal toxicity [1, 2]. Individual decisions may be taken.

Anthracyclines, cyclophosphamide and taxanes, the standard adjuvant or neoadjuvant combination recommended for non-pregnant patients, are recommended for treatment of BCP, after the first trimester [42–44]. One of the most widely used regimens, which is also used during pregnancy, is epirubicin/cyclophosphamide (EC) followed by weekly paclitaxel (EC-Pw). The reverse sequence, starting with a taxane, is also possible [45]. Currently, the data do not support the use of anthracycline- or taxane-free regimens, as these are not considered to be standard in non-pregnant women. It was found that 5-fluorouracil does not add any benefit to an anthracycline-taxane-based regimen [46] and is therefore no longer indicated for breast cancer therapy.

Platinum derivatives may have a role in triple-negative breast cancer patients [47]. Neoadjuvant trials demonstrated significantly higher pathological complete response rates by adding carboplatin, but data are immature for survival analyses. Therefore, carboplatin may be considered during the second and third trimesters of pregnancy [48, 49]. It is unclear which platinum molecule is most effective, but carboplatin may have less overall toxicity than cisplatin [38].

Several studies have shown that dose-dense (same dose administered over a shorter interval) or intensified dose-dense (IDD; higher dose over a shorter interval) treatment leads to better survival than conventionally dosed chemotherapy regimens, especially in high-risk patients [50, 51]. While dose-dense chemotherapy seems to be an acceptable option during pregnancy, IDD chemotherapy has not been studied systematically, and only a small number of reports are available [3]. The high rate of grade 2–4 anaemia (59%), with a need for transfusion in 28% of patients, and the high risk of febrile neutropenia (7% despite primary granulocyte colony-stimulating factor (G-CSF) prophylaxis) mandate a very strict risk/benefit analysis, and IDD treatment can therefore not generally be recommended in BCP.

59.7.1.1 Special Considerations in Pregnancy

General rules for administering chemotherapy to a pregnant breast cancer patient are summarised in Table 59.3.

The physiological variations in drug pharmacokinetics during pregnancy raise important concerns regarding optimal drug dosing in pregnant patients [52]. Physiologic alterations associated with pregnancy result in lower maximal concentrations of chemotherapy and a lower area under the concentration-time curve [53]. Most anticancer agents are empirically prescribed according to body surface area (BSA), resulting in large inter-patient variability, even outside the pregnancy setting.

An increased activity of major enzymes involved in the metabolism of taxanes and anthracyclines (including cytochrome p450 isoforms such as CYP3A4 or CYP2C8) has been observed during the late trimesters of pregnancy, potentially resulting in decreased drug exposure [54]. Moreover, since albumin concentrations vary significantly during pregnancy and taxanes are highly protein bound, this may lead to significant changes in taxane pharmacokinetics [52]. Pharmacokinetic data comparing the use of anthracyclines and taxanes in pregnant versus non-pregnant patients demonstrated that taxane serum levels were significantly decreased during pregnancy, especially for paclitaxel [55]. Conversely, exposure to anthracyclines was not significantly modified by pregnancy [52, 53].

Table 59.3 General rules for safe application of chemotherapy during pregnancy

Rule	Comment
Maintain dose intensity	Important to discuss timing of the chemotherapy start in relation to delivery
Use published standard protocols	Neither decrease nor increase the dose Do not increase treatment intervals
Dose according to actual bodyweight	Important to avoid underdosing, which is a risk factor during pregnancy, due to physiologic variation in drug pharmacokinetics. We do not recommend dose adaptation in overweight non-pregnant women
Do not increase the dose	Some data show a lower AUC and C_{max} in women with taxanes treated during pregnancy vs non-pregnant women. Based on 11 cases without outcome data, dose increase cannot be recommended
Recommended to stop chemotherapy around 35th to 37th week of gestation	To allow the bone marrow to recover and prevent hematologic toxicity to the mother and child

AUC area under the concentration-time curve, C_{max} maximum serum concentration

Whether doses should be increased in pregnancy remains uncertain, given that such increases could result in severe toxicities, with potential harm for mother and neonate. Secondly, in overweight women, who also have altered pharmacokinetics, the dose will not be increased [56]. Thirdly, it was shown that the chemotherapy is as active in pregnant as in non-pregnant women [57]. Thus, dosing based on BSA, using the current patient weight (prior to every course), remains a standard, as well as using the same dose for pregnant as non-pregnant women.

While maternal drug exposure is a concern in terms of treatment efficacy, the transplacental transfer of anticancer agents is a critical issue for foetal safety. The placenta is the central organ for foetal-maternal exchange, in addition to its functions such as the protection of the foetus and preparing the maternal body for pregnancy and subsequent lactation [58]. While the transplacental transfer of pharmaceuticals can be well analysed using the perfused human ex vivo placenta, toxic effects of cancer therapy on the human placenta are poorly understood, and data are limited [59]. One reason for this might be that most animal models fail to represent central features of human placentation, with even closely related species such as rhesus monkeys show diverging invasion patterns [60].

Data on transplacental transfer rates indicate similar and reassuring data on anthracyclines and taxanes, although with marked inter-patient variability, particularly with docetaxel [11]. As a consequence, from the foetal safety point of view,

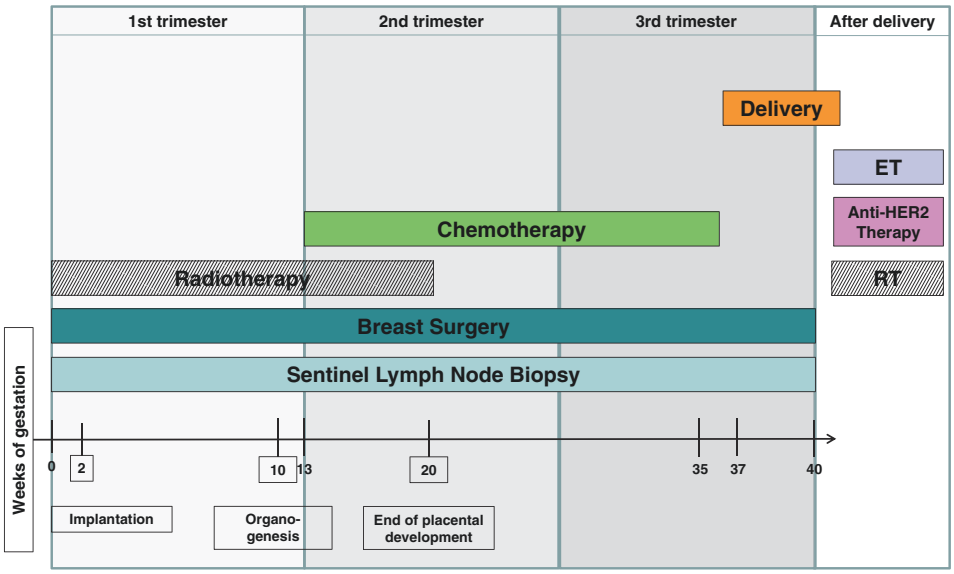

Fig. 59.2 Overview of therapeutic options during pregnancy. Crucial phases: implantation (0–2 w), organogenesis (2–10 w), foetal phase (>10 w). Starting chemotherapy from week 13 to 14 instead of week 10 allows a 'safety period'. Radiotherapy if indicated and decided not to be postponed after delivery can be applied during the first until early second trimester. Preferred option if possible to apply after delivery. Endocrine therapy and anti-HER2 treatment to be given after delivery

paclitaxel should probably be preferred to docetaxel in the setting of pregnancy [61]. Significant transplacental transfer of carboplatin was demonstrated, but long-term data from children remain limited [38, 62].

A significantly higher incidence of small-for-gestational-age babies is observed when chemotherapy is given during pregnancy, indicating a potentially toxic influence on placental development leading to placental malfunction, e.g. via incomplete trophoblast invasion into the uterus, resulting in a decreased transfer of nutrients to the foetus [10, 63]. Organogenesis is completed around the tenth week of gestation (and this is the reason why chemotherapy can be considered from that time point onwards); trophoblast invasion of the placenta is not completed until around week 20 [64]. These observations explain why starting chemotherapy at week 14 might interfere with late stages of placental development (Fig. 59.2).

59.8 Anti-HER2 Treatment

Trastuzumab is indicated as an integral part of primary treatment in women with HER2+ breast cancer. Initiating trastuzumab as early as possible, and in combination with the cytotoxic agents rather than in sequence, is associated with a better long-term outcome in non-pregnant patients [65]. In a recent review, the authors identified 18 reports in the literature of using trastuzumab during pregnancy and 19 newborns [43]. They described oligo- and anhydramnios as the most frequent side effect (33.3%) that was in general self-limiting when trastuzumab was stopped. However, most of the pregnancies ended prematurely, and four of the newborns died due to complications of prematurity (mainly respiratory failure). Although it is generally not recommended to use trastuzumab during pregnancy, it may be discussed in special high-risk situations [43]. Foetal and maternal risks and benefits need to be weighed, and informed decision-making is absolutely crucial, if trastuzumab is considered for use during pregnancy. However, inadvertent foetal exposure of 1–2 cycles of trastuzumab is no reason for termination of pregnancy. Pertuzumab in addition to trastuzumab and chemotherapy increases the pathological complete response rate in patients with HER2+ breast cancer, but currently there are no data on the use of pertuzumab during pregnancy.

59.9 Supportive Treatment

The overall aim is to offer the best supportive therapy without adding further risk. In general the majority of supportive regimens can be given safely during pregnancy (Table 59.4). According to international guidelines, women receiving an anthracycline/cyclophosphamide combination are at particularly high risk of nausea and vomiting. A 3-drug regimen including a 5-HT3 receptor antagonist, dexamethasone and a neurokinin1 (NK1) inhibitor is recommended in non-pregnant patients [66]. 5-HT3 antagonists have been extensively studied for pregnancy-induced and spinal anaesthesia-induced nausea and vomiting and were shown to be safe [67]. The use of NK1 inhibitors, without adverse effects, has been reported only in single cases of a BCP registry (GBG data on file), but these agents cannot be recommended until more safety data become available. The recommendation on dexamethasone remains unchanged (Table 59.4) [68–70].

Although granulocyte colony-stimulating factor (G-CSF) support can reduce the occurrence of febrile neutropenia, its effectiveness and safety profile during pregnancy are not clearly confirmed. One retrospective analysis reported that the use of daily or long-acting G-CSF did not affect outcome in the newborn [71]. As dose-dense chemotherapy absolutely requires the use of primary prophylaxis with G-CSF, this supportive treatment should not be withheld if a careful risk/benefit assessment indicates that this more aggressive form of chemotherapy is required.

Table 59.4 Supportive therapy for chemotherapy during pregnancy

Drug class	Examples	Recommendation
Antiemetics		
5-HT3 antagonists	*Ondansetron* Palonosetron Granisetron Tropisetron Dolasetron	Ondansetron during pregnancy not associated with significantly increased risk of adverse foetal outcomes. Other 5-HT3 antagonists are less well investigated. Granisetron does not appear to cross the placenta
Neurokinin 1 inhibitors	*Aprepitant* Fosaprepitant	No data available; single reports with no adverse outcome can be given if necessary
Corticosteroids	Dexamethasone Betamethasone *Methylprednisolone*	Dexamethasone contraindicated in the first trimester (risk of cleft palate). Attention deficit disorder reported with dexa- and betamethasone use. Methylprednisolone is the preferred option
H1 antagonists		Seem to be safe
H2 antagonists	*Ranitidine* *Cimetidine*	No increased incidence of malformations with H2 blocker. Can be used to prevent allergic reaction
Proton pump inhibitors	Omeprazole Pantoprazole	Seems to have muscle-relaxant effects in vitro
Colony-stimulating factors		
Granulocyte-stimulating colony factor	*Daily use* (*filgrastim*, lenograstim) or *long acting* (pegfilgrastim, lipegfilgrastim)	Information about the use of G-CSF during pregnancy is limited. In a series of 34 children exposed to daily G-CSF, no splenomegaly and no increased rate of opportunistic infections were reported

5-HT3 5-hydroxytryptamine, *H1* histamine H1 receptor, *H2* histamine H2 receptor, *G-CSF* granulocyte-stimulating colony factor

59.10 Obstetrical Care

In utero exposure to chemotherapy has also been associated with a small increase in risk of preterm rupture of membranes (3% vs. 0%) and preterm labour (6% vs. 2%) [10]. The largest and most recent studies report a mean gestational age at delivery of 36–37 weeks, indicating that a significant proportion of patients deliver (iatrogenically) preterm [10, 63]. Reassuring data from older studies investigating long-term outcome of children antenatally exposed to chemotherapy were recently confirmed using a standardised age-appropriate assessment to examine neurocognitive functioning, as well as in a subsequent case-control study [72, 73]. Since prematurity has an important impact on neuropsychological outcome, this should be avoided whenever possible. Treatment during pregnancy may help to achieve a full-term pregnancy. Also, the cardiac outcome of children who received anthracyclines antenatally appears to be reassuring [74].

The obstetrician should see the patient at least once every 3 weeks with an ultrasound assessment of the foetus, the amniotic fluid and the flow in the umbilical artery, in addition to standard prenatal care. Prior to start of treatment, the status quo of the pregnancy should be documented and the estimated date of delivery confirmed. If the pregnancy is complicated, for example, by gestational diabetes or hypertension, additional measures need to be implemented, and shorter intervals might be necessary.

It is recommended to deliver as closely as possible to term, after close observation of the mother and child. A 2–3-week interval between the last chemotherapy cycle and delivery is recommended, in order to allow the bone marrow to recover and prevent hematologic toxicity to the mother and child.

Conclusion

Breast cancer should be treated during pregnancy following the general guidelines for young non-pregnant patients as closely as possible. The complex medical situation of breast cancer in pregnancy requires a multidisciplinary discussion. Major concerns are congenital malformations, effects on foetal growth, preterm delivery and long-term toxicity in children [10]. An individual risk/benefit analysis, taking into account the mother and foetus, is crucial. Staging and treatment procedures need to be discussed, aiming to reduce the foetal toxicity from (accumulated) radiation. A close collaboration with the obstetrician and perinatologist is warranted.

Evidence for current and future recommendations for BCP, taking into account treatment evolvement for the care of our patients, can be generated only by large prospective cohort studies. BCP cases should be registered through the German Breast Group (www.germanbreastgroup.de) or through the registry of the INCIP (www.cancerinpregnancy.org). These international collaborations started 10 years ago and have provided the basis of current knowledge on BCP.

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Chest Wall Disease: The Clinical Continuum Between Inflammatory and Lymphangitic Breast Cancer

60

Giuseppe Curigliano

60.1 Introduction

Inflammatory breast cancer (IBC) is an uncommon entity that affects about 2.0–2.5% of women diagnosed with breast cancer [1]. The clinical presentation consists of diffuse erythema, rapid enlargement of the breast, skin ridging, and a characteristic "peau d'orange" appearance of the skin secondary to dermal lymphatic involvement [2, 3]. Overall survival is shorter than with non-IBC [4, 5]. Many patients relapse and progress locally to a lymphangitic spread to chest wall and to metastatic disease. Lymphangitic breast cancer (LBC) is pathologically characterized by high vascularity, skin lymphatic vessel infiltration, and increased microvessel density because of high expression of angiogenic factors [3]. Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis and is involved in endothelial and tumor cell growth and motility and blood vessel permeability [6]. Extensive vascular involvement of LBC makes this tumor especially amenable to antiangiogenic treatment. The use of bevacizumab, a VEGF-targeting monoclonal antibody, resulted in improved progression-free survival and response in patients with advanced breast cancer in several randomized phase III trials [7–12].

60.2 Management of Inflammatory Breast Cancer

The diagnosis of IBC should remain a clinical one with essential pathological confirmation of invasive carcinoma, while dermal lymphovascular tumor emboli, when a skin punch biopsy is carried out, is pathogneumonic but not required for a diagnosis. Although routine breast radiological investigations are recommended as part of staging workup, the data are currently not sufficient to define any radiological signs specific for IBC and are therefore not part of the diagnostic criteria. A multidisciplinary approach for women with IBC is recommended. Primary systemic chemotherapy, surgery, and radiation therapy should all be included in the treatment plan. Due to the fact that most women with IBC will have locoregional disease at presentation and the presence of extensive skin involvement, surgery should not be considered the first approach. If surgery is attempted upfront, the probability of residual disease being left behind is high, and therefore, it is strongly recommended that patients with clinical diagnosed IBC be referred to a medical oncologist. All women with IBC should be offered primary systemic chemotherapy as the first line of treatment with the goal of downstaging the tumor to allow for definitive surgery. There are no data from large randomized clinical trials looking at the optimal chemotherapeutic regimen specifically for women with IBC. Thus, recommendations made are based primarily on retrospective studies, small prospective studies, and extrapolation of data available from prospective trials evaluating women with non-IBC tumors. Systemic treatment should be defined according to biological features of disease. The monitoring of response to primary systemic chemotherapy should be a combination of physical examination and radiological assessment. Physical examination of the breast for response may be conducted at every course of systemic therapy. Radiological assessment should be carried out at the end of treatment and compared with baseline results. Due to clinical presentation, the physical examination and imaging techniques can underestimate the extent of residual disease. Despite a clinical response to treatment is observed, residual disease may still be present in the affected skin of the involved breast. The surgical approach to IBC following preoperative systemic treatment is a modified radical mastectomy. A skin-sparing mastectomy approach is contraindicated, and breast-conserving approaches may only be attempted within the context of a clinical trial. All women with IBC who undergo a modified radical mastectomy are recommended to receive postmastectomy radiation therapy. Since a

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high probability exists of involvement of locoregional lymph nodes, which would predict for a high likelihood of locoregional recurrence, is highly recommended radiation therapy on the supraclavicular regions and internal mammary lymph nodes. It is also recommended that the cumulative radiation dose be escalated to 66 Gy in the subset of women who are <45 years of age, who have close or positive surgical margins, who have four or more positive lymph nodes following preoperative systemic treatment, or who have demonstrated a poor response preoperative systemic treatment. Skin dose should be modulated to ensure moderate acute erythema in response to radiation.

60.3 Clinical and Pathological Characteristics of Inflammatory Breast Cancer

A differentiation between primary and secondary inflammatory breast cancer has to be made. By primary inflammatory breast cancer, we refer to the development of breast carcinoma in a previously normal breast. The term secondary inflammatory breast carcinoma is given to the development of inflammatory skin changes associated with invasive breast carcinoma in a breast that already had cancer, or there was carcinoma in the chest wall that developed after a mastectomy for noninflammatory breast carcinoma. Several conditions can mimic the clinical presentation of IBC. Non-puerperal bacterial mastitis may be confused with IBC, leading to potentially preventable delays in diagnosis and treatment. The skin changes in IBC are caused by tumor emboli within the dermal lymphatics and—contrary to the suggestion evoked by the nomenclature—not by infiltration of inflammatory cells. Although microscopical detection of tumor emboli in dermal lymphatic vessels is supportive of the diagnosis, it is not required. Furthermore, dermal lymphatic invasion without typical clinical findings is not sufficient for a diagnosis of IBC.

60.4 Epidemiologic Features

Inflammatory breast cancer is the most aggressive entity of breast cancer and comprises 2.5% of all breast cancers [1]. The median overall survival among women with IBC is less than 4 years even with multimodality treatment options. However, an increasing survival in recent years has been noted with improvement of chemotherapeutical management [2]. The incidence of IBC appears to be increasing, particularly among Caucasian women. Women with IBC typically present at a younger age than non-IBC [2]. Four large population-based studies have reported a higher incidence in young African-American women, and they had a worse survival compared to Caucasian women. The cause of racial disparities has not yet been elucidated [2–5]. It has been noted that Hispanic women had the youngest mean age of onset (50.5 years) compared with 55.2 years for African-American women and 58.1 years for Caucasian women [2]. Data on risk factors is limited: a high body mass index (BMI) is positively associated with a diagnosis of IBC compared to non-IBC [2]. Several other risk factors have shown some indication of being associated with the diagnosis of IBC (e.g., younger age at live first birth), but further studies are warranted [2]. In contrast, higher level of education was associated with reduced risk of ER-positive IBC, more so than for noninflammatory breast cancer. Advanced age at first birth was associated with reduced risk of ER-negative IBC. Several studies have reported that IBC constitutes a larger proportion of breast cancers in low-income countries than Western countries [13, 14]. Managing IBC in low-income countries poses a different set of challenges including access to screening, stage at presentation, adequacy of multidisciplinary management, and availability of therapeutic interventions [15].

60.5 Biological Features of Disease

IBC is characterized by less hormone receptor expression compared to noninflammatory breast cancer (NIBC), which has been associated with a more aggressive clinical course and decreased survival [16, 17]. Up to 83% of IBC tumors lack estrogen receptor (ER) expression compared with other forms of locally advanced breast cancers which are mostly ER positive [18, 19]. Analysis of 2000 patients with IBC from the California Cancer Registry has shown that expression of ER and PR was lower among IBC patient cases compared to both non-T4 carcinomas (56% ER, 45% PR versus 80% ER, 68% PR) and in patients with locally advanced breast cancer (67% ER, 54% PR) [19]. Despite a decreased estrogen receptor expression in IBC, hormone production might still play a role. GPR30 expression (a seven-transmembrane receptor belonging to the G protein-coupled receptor family and regulates cellular and physiological responsiveness to estrogen) was found in 69% of patients with IBC which was not interdependently expressed with ER. Therefore, estrogen signaling may be active in ER-negative IBC patients [20]. Subsequently, it may be possible to exploit new potential therapies through nonclassical estrogen-dependent pathways despite the lack of detectable ER. Specific GPR30 antagonists (G15 and G36) have shown to inhibit estrogen-stimulated proliferation of uterine epithelial cells in vivo. Further assessment of the effects and mechanisms of action of both agents in IBC cell lines and tumor xenografts is yet to be conducted [21, 22].

60.6 Epidermal Growth Factor Receptors

The epidermal growth factor receptor family plays an important role in cell proliferation, survival, migration, and differentiation and consists of four members: epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2, 3, and 4 (HER2, HER3, and HER4) [23]. EGFR overexpression was detected in 30% of patients with IBC and found to be associated with a significantly worse 5-year overall survival rate compared to EGFR-negative IBC. Furthermore, EGFR expression was associated with increased risk of IBC recurrence [23]. In an IBC xenograft model, erlotinib (an EGFR tyrosine kinase inhibitor) inhibited IBC tumor growth and inhibited spontaneous lung metastasis. These results suggest that the EGFR pathway is involved in tumor growth and metastasis of IBC and thereby potentially represents an effective therapeutic target [24]. Human epidermal growth factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase and is involved in signal transduction pathways leading to cell growth and differentiation [25]. Overexpression of HER2 in breast cancer is associated with increased aggressiveness and higher recurrence rates and higher mortality [26]. IBC patient cases were noted to have a higher proportion of HER2-positive patient cases compared with non-T4 patients and compared with LABC [27]. Despite the association with advanced tumor stage, HER2-positive status is not an independent adverse prognostic factor for survival among IBC patient cases [27]. The NOAH trial aimed to assess event-free survival in patients with HER2-positive locally advanced or inflammatory breast cancer, respectively, 144 and 77 patients, receiving neoadjuvant chemotherapy with or without 1 year of trastuzumab. The addition of neoadjuvant and adjuvant trastuzumab to neoadjuvant chemotherapy showed a significantly improved event-free survival in patients with HER2-positive breast cancer (3-year event-free survival of 71% study) and a significantly improved pathological response in both breast tissue and axillary lymph nodes [22]. When trastuzumab is administered in the neoadjuvant setting only, with an average of 20 weeks preoperative administration, patients with IBC continue to have a high risk of locoregional recurrence and relatively early recurrence in the brain even when pathological complete response is reached [27]. However, it should be noted that no comparison with NIBC was made and that current standard is 1-year duration of trastuzumab treatment, rather than 20 weeks only. Lapatinib is a dual inhibitor of the EGFR and HER2 receptor tyrosine kinases. Lapatinib induces tumor delayed cell growth or apoptosis in EGFR- or HER2-dependent tumor cell lines or xenografts [49]. A phase II trial was performed to investigate the neoadjuvant administration of lapatinib in combination with paclitaxel [28]. Patients were assigned to cohorts A (HER2-overexpressing [HER2+] ± EGFR) or B (HER2−/EGFR+). The primary end point was pathologic response, which was evaluated at the time of surgical resection at the completion of 12 weeks of lapatinib/paclitaxel combination therapy and was defined according to evidence of residual invasive tumor, including residual tumor in the axillary lymph nodes. The HER2-negative/EGFR-positive cohort had been terminated because of lack of efficacy observed in another trial with IBC patients with HER2-negative/EGFR-positive tumors. Secondary endpoints included safety and tolerability of lapatinib and paclitaxel combination [27]. A neoadjuvant treatment regimen of daily lapatinib monotherapy for 14 days, followed by combination therapy with daily oral lapatinib and weekly paclitaxel for 12 weeks, had a combined clinical response rate of 78.1% in IBC patients with HER2-overexpressing tumors without unexpected toxicity [29]. The impact on DFS and OS of neoadjuvant administration of lapatinib has to be evaluated in future clinical trials. Remarkably, HER3 has been identified as a potential marker of drug sensitivity in lapatinib therapy [30]. Phosphorylated HER3 predicted response to lapatinib and tumors coexpressing phosphorylated HER2 and HER3 were more likely to respond [30]. As a prognostic marker, expression of HER3 has been associated with reduced breast cancer-specific survival [31]. A more complete picture of the role of HER3 as a therapeutic target or potential marker in IBC is yet to emerge. HER3 lacks a tyrosine kinase domain; therefore other potential targets than the tyrosine kinase domain have to be addressed. Several ligands, such as the neuregulins and heregulin, bind HER3 [32, 33]. Blocking heregulin expression inhibits tumorigenicity and metastasis of breast cancer cells [34]. HER3 ligands could thereby be potential therapeutic targets in IBC.

60.7 Tumor Suppressor Genes and Oncogenes

Tumor suppressor p53 is a transcription factor that regulates the cell cycle. Alteration or inactivation of p53 by mutation can lead to cancer development [34]. Higher levels of dysregulated p53 expression have been detected in IBC compared with other locally advanced breast cancers, however not statistically significant: 53% versus 36% ($p = 0.19$) [35]. In a study of 24 patients, it was shown that patients with IBC with a p53 gene mutation and nuclear overexpression of p53 protein have an 8.6-fold higher risk of death compared with patients that had neither mutation nor protein overexpression. Moreover, an important prognostic interaction with ER expression was observed. Patients who were both ER negative and had nuclear p53 overexpression had a 17.9-fold higher risk of death, compared to 2.8-fold for women with tumors that had p53 nuclear overexpression alone [36]. Analysis of 95 patients with IBC has shown that patients with IBC who do not have dysfunctional p53 protein

expression (p53 negative) have a better prognosis compared to p53-positive IBC when treated with optimal systemic and locoregional treatments. All recurrences and deaths in this study, 28 and 26, respectively, occurred in the group of nuclear p53-positive tumors [37]. As p53 status seems to have an important influence on outcome, the results of the INGN-201-bioengineer construct are eagerly awaited. INGN-201 is an adenoviral vector that carries the normal p53 gene under the control of the cytomegalovirus (CMV) promoter. INGN-201-mediated p53 expression induces apoptosis and/or inhibition of proliferation in vitro in cancer cell lines from numerous tumor types, with almost no effects on normal cells [38]. INGN-201 was investigated in combination with docetaxel and doxorubicin in locally advanced breast cancer [38]. Unfortunately, no results are known and patients with IBC were excluded. However, the higher levels of expression of p53 IBC cancer may justify the use of INGN-201 in future IBC trials. Another potential target might be anaplastic lymphoma kinase (ALK) genetic abnormalities. ALK is a receptor tyrosine kinase (RTK) within the insulin receptor superfamily, and there has been evidence for the activation of ALK pathway activation in preclinical models of IBC [39]. Crizotinib, a small molecule ALK inhibitor, showed promising results in non-small cell lung cancer patients with ALK genetic abnormalities compared with standard second-line chemotherapy [40]. Crizotinib arrested growth of IBC cells in culture and activated the cell death pathway [39]. Based on these results, IBC patients are being screened for ALK genetic abnormalities and, if eligible, included in clinical trials with ALK inhibitors [41].

60.8 (Lymph) Angiogenic Factors

The dependence of solid tumors on blood supply for their ability to grow and metastasize is nowadays an established concept in tumor biology. Tumor angiogenesis, the sprouting of new capillaries from existing vessels, is the result of a complex and precise balance between proangiogenic and antiangiogenic factors and is essential to the growth of primary and metastatic tumors beyond the diameter of 1–2 mm^3. A variety of endogenous factors associated with angiogenesis induction have been studied extensively, including vascular endothelial growth factors (VEGF) and basic fibroblast growth factors (bFGF). Recently, endogenous inhibitors of angiogenesis gained more attention. At time of diagnosis, most patients with IBC have axillary lymph node involvement [63]. Lymphatic metastases can occur by invasion of pre-existing lymph vessels and by tumor-induced lymph angiogenesis in which VEGF also plays an important role [42]. Therefore, it might be an interesting molecular mechanism to target in the prevention of axillary involvement. Molecular and histomorphometric studies of human IBC samples have provided evidence of increased angiogenesis and lymphangiogenesis in IBC. Significant increased intratumoral microvessel density was observed in IBC patients compared to NIBC, thereby indicating IBC as a highly vascular disease with an enlarged intratumoral vascular area [43]. Furthermore, a positive correlation between the expression of carbonic anhydrase IX (an endogenous hypoxia marker) and endothelial cell proliferation was found. However, expression of CA IX was significantly less frequent in IBC than in NIBC with early metastasis. There was a significant positive correlation between the expression of CA IX and endothelial cell proliferation in IBC, implying that the angiogenesis is partly hypoxia driven. However, the higher endothelial cell proliferation in IBC and the less frequent expression of CA IX in IBC versus NIBC points at a role for other factors than hypoxia in stimulating angiogenesis [44]. Molecular evidence of increased angiogenesis was provided by elevated mRNA expression of angiogenic factors and their receptors which were quantified by real-time reverse transcriptase polymerase chain reaction (RT-PCR). Among others, expressions of TIE-1 and TIE-2 (cell surface proteins of endothelial cells), which have been described in angiogenesis, are elevated [45]. Histomorphometric evidence of lymphangiogenesis appeared from a study comparing samples from 29 patients with IBC with 56 samples from patients with NIBC. A higher lymphatic endothelial cell proliferation in IBC was demonstrated and a larger relative tumor area occupied by lymph vessels compared to NIBC [46]. As previously noted, VEGF is involved in both angiogenesis and lymphangiogenesis, and elevated levels of VEGF are found to be highly expressed in IBC [2, 47]. It was observed that intratumoral VEGF-C and VEGF-D mRNA were significantly more expressed in IBC than in patients with noninflammatory disease [47]. VEGF-C has shown to be associated with increased lymph vessel density and lymph node involvement in invasive breast cancer [48]. VEGF-D can induce both tumor angiogenesis and lymphangiogenesis and promotes the lymphatic spread of tumors [45]. By real-time quantitative reverse transcriptase-PCR, levels of mRNA of tumor angiogenesis and lymphangiogenesis-related factors (e.g., VEGF) were measured in 16 patients with IBC and 20 patients with noninflammatory breast cancer. No significant difference in expression level of angiogenic VEGF-A in inflammatory breast cancer was found when compared with noninflammatory breast cancer. However, its receptor (vascular endothelial growth factor receptor 2) was significantly upregulated in IBC versus noninflammatory breast cancer. VEGFR-2 is predominantly expressed in endothelial cells, and its activation results in a mitogenic and migratory response. Most functions of VEGF are mediated through this receptor [46]. Furthermore, it was demonstrated that tumor stromal VEGF-A expression is a valuable

prognostic indicator of breast cancer-specific survival and disease-free survival at diagnosis and can therefore potentially be used to stratify IBC patients into low-risk and high-risk groups for death and relapses [48]. In a retrospective analysis, IBC samples were compared to normal breast tissue from reduction mammoplasty patients. Significantly lower epithelial VEGF-A immunostaining was found in IBC tumor cells than in normal breast tissues, cytoplasmic VEGF-R1 and nuclear VEGF-R2 levels were slightly higher, and cytoplasmic VEGF-R2 levels were significantly higher ($P = 0.04$). Sixty-two percent of IBC tumors had high stromal VEGF-A expression. Stromal VEGF-A levels predicted breast cancer-specific survival (BCSS) and DFS in IBC patients with estrogen receptor positive ($P < 0.01$ for both), progesterone receptor positive ($P = 0.04$ and $P = 0.03$), HER2+ ($P = 0.04$ and $P = 0.03$), and lymph node involvement ($P < 0.01$ for both). Tumor stromal VEGF-A was identified as an independent predictor of poor BCSS (hazard ratio [HR], 5.0; 95% CI, 2.0–12.3; $P < 0.01$) and DFS (HR, 4.2; 95% CI, 1.7–10.3; $P < 0.01$). This might indicate that tumor stromal VEGF-A expression is a valuable prognostic indicator of BCSS and DFS at diagnosis and can therefore be used to stratify IBC patients into low-risk and high-risk groups for death and relapses. High levels of tumor stromal VEGF-A may be useful for identifying IBC patients who will benefit from antiangiogenic treatment. Due to the displayed highly angiogenic features, patients with IBC might benefit from antiangiogenic agents that target VEGF [2]. Bevacizumab is a monoclonal antibody to VEGF and has been shown to inhibit VEGF receptor activation, specifically VEGF-A [2, 42]. However, bevacizumab's indication to treat locally recurrent or metastatic HER2-negative breast cancer has been removed by the Food and Drug Administration (FDA). It stated that no trial in breast cancer using bevacizumab provides evidence of direct clinical benefit and that only modest effects on primarily radiographic outcomes were demonstrated. These modest indirect measures of clinical benefit must be weighed against a marked increase in clinically serious adverse events (gastrointestinal perforations, hemorrhage, surgery, and wound healing complications) and therapy-related deaths. Deaths attributed to bevacizumab ranged between 0.8% and 1.2% as released by the Food and Drug Administration [49]. Despite these concerns involving treatment of breast cancer with bevacizumab, there may still be an indication for a subgroup of patients. For example, since IBC is more angiogenic than noninflammatory breast cancer and has significantly higher levels of VEGF expression, bevacizumab treatment may be useful in IBC patients [43]. Some hints for efficacy of bevacizumab in IBC have been suggested in small clinical studies. The first study demonstrated a significant decrease of 66.7% in phosphorylated VEGFR-2 in 21 patients with inflammatory and locally advanced breast cancer (one patient had NIBC since the study briefly was open to NIBC patients) which were treated with one cycle of bevacizumab, followed by six cycles of bevacizumab with doxorubicin and docetaxel. However, clinical benefit in terms of DFS and OS has not been determined [50]. In another study, 20 patients with IBC and one with locally advanced breast cancer received one cycle of bevacizumab followed by six cycles of bevacizumab with docetaxel-doxorubicin before surgery. Angiogenic markers were measured at baseline before bevacizumab, after bevacizumab, and after bevacizumab plus chemotherapy. VEGF-A was higher at baseline in the responders than nonresponders, demonstrating a trend toward association with response. Moreover, baseline CD31 and platelet-derived growth factor (PDGFR) beta were significantly associated with response to bevacizumab. Patients with IBC with higher tumor gene expression of VEGF-A, CD-31, and PDGFR-beta were more likely to benefit from treatment with bevacizumab with chemotherapy [50]. In a phase II, multicenter, open-label, single-arm, noncomparative trial, patients with histologically confirmed HER2-positive nonmetastatic IBC were enrolled to assess efficacy and safety of neoadjuvant bevacizumab combined with trastuzumab and chemotherapy. Primary end point was pathological complete response. Before surgery, patients were treated with fluorouracil, epirubicin, cyclophosphamide, and bevacizumab (cycles 1–4) and docetaxel, bevacizumab, and trastuzumab (cycles 5–8) in 3-week cycles. After surgery, patients received adjuvant radiotherapy, trastuzumab, and bevacizumab. After neoadjuvant therapy, 33 of 52 patients had a pathological complete response. Furthermore, this treatment regimen seemed to be well tolerated [51]. In another prospective, phase II randomized trial, oral vinorelbine plus capecitabine and bevacizumab (BEVIX) is an active regimen for patients with LBC [52]. Patients with inflammatory or LBC have a poorer prognosis than those with other breast cancers. In the phase II BEVERLY 2 study, women with histologically confirmed HER2-positive nonmetastatic IBC were treated with fluorouracil, epirubicin, cyclophosphamide, and bevacizumab (cycles 1–4) and docetaxel, bevacizumab, and trastuzumab (cycles 5–8) in 3-week cycles [51]. After neoadjuvant therapy, 33 of 52 patients had a pathological complete response [51]. In the phase II PEGASE 02 study, patients with IBC received neoadjuvant chemotherapy with cyclophosphamide, doxorubicin, and fluorouracil [53]. Although the clinical response rate was high (90%), pCR was achieved in only 32% of patients [53]. The AVEREL study evaluated first-line bevacizumab-containing therapy for HER2-positive locally recurrent/metastatic breast cancer [54]. In this study, high baseline plasma VEGF-A concentrations were associated with larger benefit of bevacizumab (not statistically significant) [54]. In another study, patients with stage III locally advanced or inflammatory breast carcinoma received four

3-weekly cycles of FEC (5-fluorouracil, epirubicin, and cyclophosphamide) followed by 12 cycles of weekly paclitaxel in combination with bevacizumab 10 mg/kg every 2 weeks as neoadjuvant therapy [55]. In the intent-to-treat population, the pCR rate was 21%, and the clinical response rate was 59% [55]. A pilot clinical trial evaluated the efficacy of neoadjuvant therapy with bevacizumab, in combination with doxorubicin and docetaxel in 21 previously untreated patients with locally advanced breast cancer, 20 of whom had IBC [11]. Tumor biopsies and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) were obtained at baseline and after cycles 1, 4, and 7. A median decrease of 66.7% in phosphorylated VEGFR-2 (Y951) in tumor cells ($P = 0.004$) and median increase of 128.9% in tumor apoptosis ($P = 0.0008$) were seen after bevacizumab alone [11]. A phase I trial investigated the efficacy of a small-molecule inhibitor of VEGFR-2, SU5416 (semaxinib), in combination with doxorubicin in 18 patients with IBC [56]. Similar to the outcome with bevacizumab, the authors observed decreased tumor blood flow after treatment, as assessed by DCE-MRI. Lymphangiogenesis is common in LBC [57]. Higher expression of lymphangiogenic factors (VEGF-C, VEGF-D, VEGFR-3, Prox-1, and fibroblast growth factor 2) is detected in LBC than in non-LBC tumor samples [11, 57]. Targeting lymphangiogenesis through the VEGF-C/VEGF-D/VEGFR-3 signaling system would be a reasonable therapeutic approach for LBC, although it will need to be further examined in both preclinical and clinical studies [58]. In the chest wall disease study with capecitabine/vinorelbine and bevacizumab, authors used LBC as a model disease to investigate biological changes associated with an antiangiogenic agent as bevacizumab [52]. The biological study on CEC, CEP, and CPP as surrogate predictive biomarkers showed that at baseline, responders had significantly higher counts of a CEC subpopulation expressing VEGFR-2 and of CPPs (possibly involved in vessel stabilization). Baseline counts of CEPs, of viable CECs, and of the inflammation-related chemokine IL-8 below the median value were associated with a significantly improved overall survival [52]. To date, no molecular feature reliably predicts the response to bevacizumab. Using DNA microarrays, they searched for multigene predictors of response in IBC with lymphangitic spread to the chest wall. They identified 16 genes that clearly separated patients that had achieved PR from those that had no response. A supervised clustering identified 75 genes involved in matrix remodeling (MMP1) and cell cycle regulation (CDKN2A). Our signature was strongly enriched for stroma that clearly highlights the importance of tumor-stroma crosstalk for progression of LBC [52]. A recent study by the World IBC Consortium generated whole-genome expression profiles of 137 IBC and 252 non-IBC (nIBC) samples [59]. They identified a 107-gene signature enriched for immunity-related genes that distinguished between responders and nonresponders in IBC. This signature was strongly enriched for immunity-related genes involved in CD8+ T-cell lymphocyte activation processes (Th1-response), suggesting a prominent role for adaptive immunity in determining response to CT in IBC [59]. The role of bevacizumab in the treatment of metastatic breast cancer is still a matter of debate. The results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-40 trial [60] and the GeparQuinto (GBG44) trial [61, 62], demonstrating an increase in the rate of pathological complete response in triple negative breast cancer following the addition of bevacizumab to neoadjuvant chemotherapy, are conflicting with the announcement by the Food and Drug Administration (FDA) on November 18, 2011, revoking approval of bevacizumab in combination with paclitaxel for the treatment of metastatic breast cancer. The unresolved issue is whether significant improvements in a surrogate end point like progression-free survival, in the absence of a benefit for overall survival, are potentially predictive of curative benefits in patients with metastatic breast cancer. The ongoing controversy surrounding bevacizumab therapy for breast cancer involves broader questions about the use of surrogate end points in clinical trials, as well as economic issues over the increasing cost of new medicines for cancer treatment. It is through this lens that the NSABP B-40 and GBG44 trials should be viewed, since each of these trials reports a significant improvement with bevacizumab in another putative surrogate clinical end point: pathological complete response. In the context of unsustainable expenditures for cancer care, comparative effectiveness research will drive mechanisms of drug approval in clinical practice, and the absence of any survival benefit of bevacizumab, or other molecularly targeted drugs, will be balanced against the considerable development costs of modern molecularly targeted oncology drugs.

60.9 Chest Wall Disease as a Balance Between Inflammation and Cancer Proliferation

A recent study by the World IBC Consortium generated whole-genome expression profiles of 137 IBC and 252 non-IBC (nIBC) samples. They identified a 107-gene signature enriched for immunity-related genes that distinguished between responders and nonresponders in IBC. This signature was strongly enriched for immunity-related genes involved in CD8+ T-cell lymphocyte activation processes (Th1-response), suggesting a prominent role for adaptive immunity in determining response to chemotherapy in IBC [60]. There is a potential role of immune-checkpoint inhibitors in treating patients with immune-therapeutic approaches. By blocking interactions between PD-L1 or PD-L2 and

PD-1, you may reactivate the immune surveillance, leading to improved antitumor activity. The programmed cell death protein-1 (PD-1) is a critical checkpoint molecule that is expressed by T cells upon activation. The PD-1 checkpoint pathway is thought to act primarily in peripheral tissues to dampen ongoing immune responses and/or to prevent damage to self-tissues. PD-1 is expressed by B cells, natural killer (NK) cells, dendritic cells, and activated monocytes, in addition to T cells. PD-1 ligands—which include PD-L1 and PD-L2, among others—are expressed by macrophages and monocytes, and these can be induced in numerous cell types in an inflammatory environment, like in lymphangitic breast cancer. Some chemotherapies may lead to immunogenic cell death resulting in activation of dendritic cells (DC) and priming of antitumor immune responses [63]. This promotion of DC maturation might also explain the capacity of some chemotherapies to reduce T regulators (Treg). In addition, as a higher frequency of proliferating cells is observed in Treg compared with the non-Treg compartment, chemotherapy, which mostly destroys proliferating cells, may tilt the balance from Treg toward effector T cells [64]. The use of metronomic cyclophosphamide (CTX) is the leading product of this therapeutic class. Reversal of immunological tolerance by CTX via inhibition of suppressor cells has been reported [65]. Selective depletion of Treg induced by CTX or other chemotherapeutic drugs requires the use of these agents at low, so-called metronomic, doses. Some studies in humans have shown improvement of T-cell effector function associated with a reduction in Treg numbers after low-dose CTX administration. We hypothesize that the use of immune-checkpoint inhibitors in combination with metronomic CTX may induce clinical response in chest wall disease.

Conclusions

Chest wall disease represents a presentation of a clinical spectrum ranging from inflammatory to lymphangitic breast cancer. Inflammation and the immune response have long been viewed as a delicate balance that has the ability to promote a durable tumor regression or promote tumor progression. Preclinical models and biomarker studies suggest that inflammatory breast cancer comprises a more important role for the tumor microenvironment, including immune cell infiltration and vasculogenesis, especially lympho-angiogenesis. Across this clinical continuum of the chest wall disease, there is an important role of the inflammation cascade. The activation of mature dendritic cells (DCs) through toll-like receptors (TLRs) or by inflammatory cytokines converts immature DCs into mature DCs that present specific antigen to T cells, thereby activating them. Maturation of DCs is accompanied by co-stimulatory molecules and secretion of inflammatory cytokines polarizing lymphocytic, macrophages, and fibroblast infiltration. It is unknown whether immune cells associated to the IBC microenvironment play a role in this scenario to transiently promote epithelial to mesenchymal transition (EMT) in these cells. Immune and microenvironment factors can induce phenotypic, morphological, and functional changes in breast cancer cells. We can hypothesize that similar inflammatory conditions in vivo may support both the rapid metastasis and tight tumor emboli that are characteristic of chest wall disease and that targeted anti-inflammatory therapy may play a role in this patient population.

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Fertility Issues in Patients with Breast Cancer or Survivors

61

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61.1 Introduction

Breast cancer is the most common malignancy in women worldwide and the most frequent tumor in female patients during their reproductive age. The incidence appears to be on the rise [1]. Approximately 5% of all breast carcinomas are diagnosed every year in women under the age of 40 [2]. The incidence is even higher in developing countries, reaching up to 20–25% in Africa and the Middle East [3].

Challenges in fertility preservation issues have acquired a growing importance over the past years. Advances in the management of breast cancer have increased survival [2], and thus more attention is being put on quality-of-life issues like chances of subsequent fertility following primary anticancer therapy. On the other hand, it is increasingly recognized that women are delaying childbearing [4], and thus more women are diagnosed with cancer before completing their families.

As recommended by the European Society for Medical Oncology (ESMO), the American Society of Clinical Oncology (ASCO), and the International Consensus Conference for Breast Cancer in Young Women (BCY2), patients should be counseled about the risk of developing treatment-induced premature ovarian failure (POF) and infertility as part of education process before systemic anticancer therapy is initiated [5–7]. All women interested in preserving fertility should be referred to fertility clinic as soon as possible to discuss the available means for fertility preservation [5–7].

61.2 Anticancer Treatments and Gonadal Function

Treatment-induced POF is a possible consequence of anticancer treatments [8]. Acute POF (i.e., occurring during treatment) may be temporary or permanent; however, women who continue to menstruate or resume menstrual function after treatment remain at risk of early menopause and significant reduction of fertility potential [9].

In breast cancer patients, the risk of developing this side effect is mainly influenced by the use of cytotoxic chemotherapy and endocrine therapy. Chemotherapy acts through a direct gonadotoxic effect [10]: cytotoxic agents cause induction of follicle and oocyte apoptosis and vascular damage to the ovaries [11, 12]. Endocrine treatments can affect ovarian reserve both directly (i.e., impairment in ovulatory and endometrial functions) and indirectly (i.e., delay to conception with subsequent ovarian aging) [13].

The key factors for the risk of developing treatment-induced POF are age of the patient at the time of treatment (i.e., older age), type and dose of chemotherapy (i.e., use of alkylating agents such as cyclophosphamide), and need of adjuvant endocrine therapy (i.e., tamoxifen; Table 61.1) [13, 14].

As shown in a large prospective observational study assessing ovarian function after breast cancer treatment, the majority of women older than 40 years had an interruption of their menstrual function after chemotherapy without recovery of bleeding in the follow-up years [15]. On the contrary, a rapid recovery of menstrual cycling was observed in patients under the age of 35 years with approximately 85% reporting normal bleeding at 6 months following the end of chemotherapy; the recovery was less pronounced for women

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Table 61.1 The risk of treatment-induced premature ovarian failure in breast cancer patients (modified from the original [14])

Degree of risk	Type of anticancer treatment
High risk (>80% risk of POF)	– CMF, CEF, CAF, TAC × 6 cycles in women aged ≥40 years
Intermediate risk (40–60% risk of POF)	– CMF, CEF, CAF, TAC × 6 cycles in women aged 30–39 years – AC × 4 cycles in women aged ≥40 years – (F)AC or (F)EC × 4 followed by T
Lower risk (<20% risk of POF)	– CMF, CEF, CAF, TAC × 6 cycles in women aged ≤30 years – AC × 4 cycles in women aged ≤40 years
Very low or no risk	– Methotrexate and fluorouracil – Tamoxifen – Trastuzumab (?)

POF premature ovarian failure; *CMF* cyclophosphamide, methotrexate, fluorouracil; *CEF* cyclophosphamide, epirubicin, fluorouracil; *CAF* cyclophosphamide, doxorubicin, fluorouracil; *TAC* docetaxel, doxorubicin, cyclophosphamide; *(F)AC* fluorouracil, doxorubicin, cyclophosphamide; *(F)EC* fluorouracil, epirubicin, cyclophosphamide; *T* taxane

aged between 35 and 40 [15]. Treatment with anthracycline-based chemotherapy (doxorubicin, cyclophosphamide [AC]) resulted in an important decrease in the proportion of patients with regular menstrual function after treatment, with a small further decline in the number of patients with menses with the addition of paclitaxel or docetaxel (T) [15]. On the contrary, cyclophosphamide, methotrexate, fluorouracil (CMF) regimen resulted in a greater proportion of patients with monthly bleeding in the first months after treatment but followed by a steady decrease in the proportion of women with menstrual bleeding in the 3 following years [15]. Finally, the use of tamoxifen resulted in a decreased proportion of women with monthly bleeding 1 year after the end of chemotherapy, although this effect became nonsignificant after 3 years [15].

In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-30 trial, more than 2000 premenopausal patients had information available on menstrual function after treatment [16]. The rate of prolonged amenorrhea at 1 year after the start of therapy was significantly different between the three treatment arms: 69.8% for sequential AC followed by T, 37.9% for AT and 57.7% for TAC [16]. The addition of tamoxifen increased the number of patients who developed amenorrhea. Approximately 61% of women younger than 40 years developed at least 24 months of amenorrhea contrasting with nearly 100% among older patients [16].

International guidelines highlight the importance of discussing with all young patients the risk of treatment-induced POF and infertility due to anticancer treatments [5–7]. Oncofertility counseling should be individualized since the risk of POF and infertility for each woman is variable [13]. Both treatment-related factors (i.e., type and dose of chemotherapy and use of endocrine therapy) and individual characteristics (i.e., age, comorbidities and ovarian reserve at baseline) should be considered in counseling these women [17]. Particularly for young breast cancer patients concerned about the possible development of this side effect, it is crucial for physicians to discuss the expected absolute benefits of the proposed anticancer treatment, such as the need for adjuvant chemotherapy or long duration of endocrine therapy in women at low risk of recurrence [13]. On the other hand, the risk of infertility should not be overestimated, and some women (e.g., very young patients with hormone receptor-negative disease) will not likely require the help of reproductive medicine after treatment [18].

61.3 Pregnancy After Breast Cancer

Approximately 50% of young breast cancer patients desire a future pregnancy at the time of cancer diagnosis [19]. However, less than 8% of these women manage to become subsequently pregnant [20]. As shown in a large population-based matched cohort study, cancer female patients have lower pregnancy rates as compared to the general population (hazard ratio [HR] 0.61; 95% confidence intervals [CI], 0.58–0.64) [21]. Among cancer survivors, women with breast tumors showed the lowest rates for subsequent pregnancies with an overall 67% reduction in the chance of having a pregnancy after cancer treatment as compared to the general population (HR 0.33; 95% CI, 0.27–0.39) [21].

This observation reflects the possible damage to ovarian reserve as a consequence of anticancer treatments but also the concerns of patients and physicians on a possible negative impact of pregnancy on breast cancer prognosis, being a hormonally driven disease. A survey investigating the attitude on fertility among oncologists and breast surgeons dealing with breast cancer showed that only 54% of participants believed that pregnancy does not affect the prognosis of breast cancer survivors and 49% of them thought that an increase in estrogen levels during pregnancy might stimulate the growth of hidden cancer cells [22].

However, the available evidence on this topic suggests that pregnancy after breast cancer does not have any negative impact on patients' prognosis and should not be discouraged, including among patients with endocrine-sensitive disease [13]. We have previously conducted a meta-analysis of 14 retrospective control-matched studies to evaluate the safety of pregnancy in women with prior history of breast cancer [23]. The study showed that women who became subsequently pregnant had a 41% reduced risk of death compared to women who did not get pregnant (pooled relative risk [PRR] 0.59; CI, 0.50–0.70) [23]. No significant differences in survival between groups were also observed

after correcting the data for the so-called "healthy mother effect" (PRR 0.85; 95% CI, 0.53–1.35) [23]. Since no specific data in patients with endocrine-sensitive disease were available, we subsequently performed a multicenter retrospective case-control study to better clarify the prognostic impact of pregnancy in breast cancer survivors according to estrogen receptor status [24]. The study showed no difference in disease-free survival between pregnant and non-pregnant survivors with both estrogen receptor-positive (HR 0.91; 95% CI, 0.67–1.24) and negative (HR 0.75; 95% CI: 0.51–1.08) disease [24]. A better overall survival was observed in the pregnant cohort (HR 0.72; 95% CI, 0.54–0.97) with no interaction according to estrogen receptor status [24]. Importantly, abortion did not show to impact patient outcome in both studies and should not be proposed for therapeutic reasons [23, 24]. Since a higher incidence of birth complications (i.e., preterm birth, caesarean section, babies with low birth weight) has been observed in breast cancer survivors [25], a close monitoring of pregnancy in these women is recommended [26].

Although pregnancy after breast cancer should not be discouraged anymore, it is not clear yet the ideal interval to wait for women to become subsequently pregnant after the end of anticancer treatments. Experts recommend to avoid early pregnancy within 2 years from diagnosis, especially in case of patients at high risk of relapse [27].

In women with hormone receptor-positive breast cancer, the use of adjuvant endocrine therapy for 5 to 10 years is an important issue to be considered [7, 28]. For this reason, it can be challenging for young survivors candidates to adjuvant endocrine therapy to initiate their childbearing plans due to the older age at the time of treatment completion and subsequent natural decline in ovarian reserve [29]. On this issue, the International Breast Cancer Study Groub (IBCSG), Breast International Group (BIG) and North American Breast Cancer Group (NABCG) have recently started a prospective international study directed to young women with endocrine-sensitive early breast cancer who desire to become pregnant and who are disease-free after 18–24 months of adjuvant endocrine therapy [30]. The POSITIVE study investigates the feasibility and safety of a temporary interruption of adjuvant endocrine therapy to allow conception [30].

The reassuring data on the safety of pregnancy after breast cancer highlights the importance of counseling young patients on the possible risk of infertility after anticancer treatments and facilitates the access of women interested in fertility preservation to the centers of reproductive medicine [5–7].

Of note, these data might increase also the number of survivors accessing fertility units after completing their anticancer treatment. To date, there is only one small retrospective study that evaluated the safety of performing assisted reproductive technology (ART) in survivors of breast cancer after the end of treatment [31]. In this study, out of 198 women who became pregnant after breast cancer, 25 patients underwent ART [31]. A total of 36 pregnancies resulted from 37 ART cycles (13 after oocyte donation, 13 ovarian stimulation for in vitro fertilization [IVF], 11 ovulation induction) [31]. Patients who underwent ART tended to have higher percentage of node-negative disease, estrogen receptor-positive and low-grade tumors [31]. With a median follow-up of 50 months, there was no difference in survival outcomes between patients with spontaneous pregnancies (16% developed cancer-related events) and those who underwent ART procedures (8% developed cancer-related events; $p = 0.54$) [31].

61.4 Strategies for Fertility Preservation

For breast cancer patients, different strategies for fertility preservation are available: embryo and oocyte cryopreservation, cryopreservation of ovarian tissue, and temporary ovarian suppression with luteinizing hormone-releasing hormone analogs (LHRHa) during chemotherapy (Table 61.2).

The choice among these options depends on several factors: patient's age and ovarian reserve at diagnosis, type of anticancer treatment planned, time available before starting treatments, and whether the patient has a partner [14].

61.4.1 Embryo and Oocyte Cryopreservation

Embryo and oocyte cryopreservation are standard strategies for fertility preservation [5, 6]. The procedure consists of performing a controlled ovarian stimulation for 10–15 days, followed by egg harvesting; the gold standard time for initiating the controlled ovarian stimulation is the early follicular phase of the menstrual cycle. For oocyte cryopreservation, unfertilized eggs are directly cryopreserved, while for embryo cryopreservation, the oocytes are fertilized using IVF procedures, and the resulting embryos are then cryopreserved. There are two different methods for cryopreservation of embryos or oocytes: slow freezing and vitrification [32]. As shown in a Cochrane meta-analysis, vitrification showed higher pregnancy rate than slow freezing (RR 3.86; 95% CI, 1.63–9.11; $p = 0.002$) [33]. Hence, vitrification has become the most applied technique, although the results are still not as high as with fresh cycles [34].

Main limitations of embryo and oocyte cryopreservation are the possible delay in the initiation of anticancer treatments (due to the need to wait the onset of menses to perform the controlled ovarian stimulation), the need for a minor surgical procedure, and the possibility to preserve fertility but not gonadal function. In some countries, embryo freezing is prohibited by law, and the strategy is not applicable in patients without a partner at the time of cancer diagnosis: in these situations, oocyte cryopreservation is the only possible technique.

Table 61.2 Main characteristics of the available strategies for fertility preservation in women with breast cancer

Type of strategy	Definition	Need for controlled ovarian stimulation	Delay to start anticancer treatment	Surgery required	Preservation of ovarian function	Preservation of fertility
Embryo and oocyte cryopreservation	Harvesting and freezing of unfertilized (oocytes) or in vitro fertilized (embryos) eggs	Yes	Yes	Yes	No	Yes
Cryopreservation of ovarian tissue	Freezing of ovarian tissue and transplantation after treatment	No	Minimal	Yes[a]	Yes	Yes
Temporary ovarian suppression with LHRHa during chemotherapy	Use of hormonal therapies to protect ovaries during chemotherapy	No	No	No	Yes	Yes[b]

LHRHa luteinizing hormone-releasing hormone analogs
[a]Two surgical procedures
[b]Limited data available

In breast cancer patients, due to the need of performing a controlled ovarian stimulation, two main concerns are raised: a possible delay in the initiation of anticancer treatments and short-term exposure to high estradiol level, both could possibly inversely impact patient prognosis.

For patients with early-stage breast cancer, evidence suggests that when the earlier adjuvant chemotherapy is administered, the better patients' outcome can be obtained [35]. Thus, to limit treatment delays for the need to wait the onset of menses, "random-start" protocols have been developed to allow to start controlled ovarian stimulation anytime during the menstrual cycle [36]. Available experience with these protocols showed comparable results in terms of retrieval of oocytes, maturation, and fertilization rates [13].

The increase in estradiol levels is probably the major concern, and thus alternative protocols for controlled ovarian stimulation have been developed for breast cancer patients. In these protocols, tamoxifen [37] or letrozole [38] is added during the stimulation phase: several studies suggested that this approach does not significantly affect the quality of the oocytes collected [37, 39], and similar pregnancy rates as those observed in infertile non-oncologic population can be obtained [40].

To date, only one prospective study evaluated the safety of performing a controlled ovarian stimulation with letrozole supplementation (COSTLES) in breast cancer patients [41, 42]. In this study, out of 337 breast cancer patients who underwent fertility counseling before chemotherapy, 120 patients elected to undergo embryo cryopreservation, while 217 did not undergo any fertility-preserving procedure and served as controls [42]. With a median follow-up of 4.9 years, there were six (5.0%) patients who developed disease recurrence in the fertility preservation cohort and 12 (5.5%) in the control group ($p = 0.86$) [42]. There was no significant difference in relapse-free survival between the two groups (HR 0.77; 95% CI, 0.28–2.13) [42]. Although with very limited numbers, the subgroup analyses according to BRCA gene mutation status ($p = 0.57$), hormone receptor status ($p = 0.75$), and timing of stimulation (before or after breast surgery: $p = 0.44$) confirmed the lack of negative impact of controlled ovarian stimulation on patients' survival outcomes [42].

The same group has recently reported also the feasibility and safety of performing two consecutive cycles of controlled ovarian stimulation with the use of letrozole before the initiation of anticancer treatments [43]. However, further research in this filed (i.e., larger number of patients and longer follow-up) is needed to confirm these findings.

Most of the available data on the success of embryo and oocyte cryopreservation derive from the infertile non-oncologic population. The age of the patient and the number of stored oocytes or embryos are crucial factors for the success of the procedures [13]. Pregnancy rate after embryo thawing ranges from more than 40% in women under the age of 35 years to less than 20% in women older than 40 years [44]. In experienced centers, similar results are observed with the use of cryopreserved oocytes [45, 46].

In cancer patients, a possible weaker response to controlled ovarian stimulation might be expected [47]. Several issues might negatively impact on the success of this procedure in the oncologic population as compared to infertile patients, mainly related to the particular protocols used (i.e., "random-start protocols" or the use of tamoxifen or letrozole) and/or the presence of a possible underlying reduced ovarian reserve at baseline (e.g., in patients with BRCA mutations) [48]. Very limited data exist on pregnancies following embryo and oocyte cryopreservation in cancer patients. A study reported fertility outcomes in 357 women who underwent oocyte cryopreservation after cancer diagnosis [49]. A total of 11 (3.1%) cancer survivors (8 with breast cancer, 1 with

endometrial adenocarcinoma, 1 with thyroid cancer, and 1 with Hodgkin lymphoma) returned for ART after the end of treatments [49]. A total of 4 pregnancies were obtained and delivered at term with no malformations in the newborns; the delivery rate per cycle was 36.6% [49]. Even more recently, Oktay and colleagues reported the fertility preservation outcomes of breast cancer patients who underwent controlled ovarian stimulation with letrozole supplementation for embryo cryopreservation within their prospective study [40]. A total of 33 women underwent 40 attempts to transfer embryos after more than 5 years from the time they underwent embryo cryopreservation: 17 women had at least one child resulting in a fertility preservation rate of 51.5% [40]. A total of 18 pregnancies were obtained resulting in 25 live births with no malformations (seven pregnancies were twins) [40]. The overall live birth rate per embryo transfer showed to be similar to that of the infertile non-oncologic population of a similar age (45.0 vs 38.2; $p = 0.2$) [40].

Despite these encouraging results, during oncofertility counseling, breast cancer patients should be aware that available data on the success of embryo and oocyte cryopreservation derive mainly from infertile non-oncologic women and that a different response to controlled ovarian stimulation cannot be ruled out [13].

61.4.2 Cryopreservation of Ovarian Tissue

Cryopreservation of ovarian tissue is an effective, yet still experimental, surgical strategy for fertility preservation. Major advantages compared to embryo and oocyte cryopreservation are the possibility to preserve both fertility and ovarian function, ovarian tissue cryopreservation causes a minimal delay in the initiation of anticancer treatment, and sexual maturity is not required. Moreover, this strategy can be performed at any time of the menstrual cycle, and no hormonal stimulation is required. However, it is an expensive technique that should be performed only in centers with the adequate expertise. Moreover, two surgical procedures are required: before the initiation of anticancer therapies, the cortical ovarian tissue is removed and then cryopreserved; after the end of treatment, the tissue is transplanted, preferably into the pelvic cavity (orthotopic site) [50].

Following successful transplantation of ovarian tissue, a rapid recovery of ovarian function (within 3–6 months) is expected in almost all cases, with possible sustained longevity of gonadal function [51, 52]. A total of 37 live births have been reported in cancer patients after transplantation of cryopreserved ovarian tissue [53]. It is hard to accurately estimate the actual pregnancy rate based on available data [54]. Recently, Donnez and colleagues combined results from four fertility centers: out of 80 women transplanted, 20 conceived resulting in a pregnancy rate of 25% [53].

It is important to note that the patient's ovarian reserve is a key factor for the success of the procedure; hence, patients older than 40 years or with reduced ovarian reserve at baseline should not be considered for cryopreservation of ovarian tissue [55].

Although this technique is still considered an experimental strategy according to major international guidelines [5, 6], it remains an option for selected patients who cannot delay the initiation of anticancer treatments [56] and in women who have already received chemotherapy [57].

Of note, it should be considered a potential risk of reintroducing malignant cells when the cryopreserved tissue is transplanted especially in patients with aggressive hematologic malignancies; however, to date, no malignant cells have been found in ovarian tissue from breast cancer patients [58].

Moreover, specific issues should be also considered in breast cancer patients carrying BRCA mutations: due to the high lifetime risk of developing ovarian cancer, bilateral salpingo-oophorectomy is generally recommended before the age of 40 years and upon completion of childbearing [59]. Due to the lack of data on the safety of this procedure in patients with BRCA mutations, cryopreservation of ovarian tissue should not be proposed to these patients [29].

Due to the relatively low number of procedures performed to date and the technical difficulties in cryopreserving ovarian tissue, referral to centers with the known expertise would be advisable [5, 6]. While the harvesting of the tissue can be performed locally, its subsequent freezing and storage should be centralized; for this reason, a well-organized network between fertility units is required [13].

61.4.3 Temporary Ovarian Suppression with LHRHa During Chemotherapy

Pharmacological protection of the ovaries during chemotherapy is an attractive option to preserve ovarian function and fertility of women candidates to cytotoxic therapy. It is easy to administer, is relatively cheap, and does not require surgery without delaying treatment initiation [29]. In addition, it can potentially protect both ovarian function and fertility [29].

The hypothesis behind the development of this technique is that the inhibitory effect of LHRHa on gonadal function may reduce the toxicity of chemotherapy on the ovaries [60]. Animal experiments in rats and monkeys supported this hypothesis showing a reduced loss of follicles during cytotoxic therapy with concurrent administration of LHRHa [61]. Several observational and phase II studies showed that this strategy was associated with resumed ovarian function in the large majority (from 70% to 100%) of treated breast cancer patients [62].

Following these promising results, several phase III studies evaluated the efficacy of this procedure. In these trials, patients with breast cancer were randomly allocated to receive (neo)

adjuvant chemotherapy with or without concurrent LHRHa [13]. These studies produced relatively conflicting results with some suggesting a protective effect while others showing no relevant impact of concurrent administration of LHRHa on reducing the incidence of chemotherapy-induced POF [13]. Of note, these studies were not identical in terms of patient population, treatment given, and definition of endpoints, which possibly contributed to the observed conflicting results.

However, despite the extensive debate on the efficacy of the procedure over the last years [63–66], in 2015, some important news on this topic have become available [67], suggesting the efficacy of temporary ovarian suppression with LHRHa for preserving ovarian function and fertility particularly in breast cancer patients [68].

Two large phase III studies reported favorable results on the efficacy and safety of the procedure [69, 70]. The POEMS-SWOG S0230 study enrolled 257 premenopausal breast cancer patients with hormone receptor-negative breast cancer, while in the PROMISE-GIM6 study, approximately 80% of the 281 included patients had hormone receptor-positive disease [69, 71]. Both studies showed that temporary ovarian suppression with LHRHa was associated with a significant reduction in the incidence of POF, 2 years after the end of chemotherapy in the POEMS-SWOG S0230 study (from 22% to 8%; odds ratio [OR] 0.30; $p = 0.04$) [69], and 1 year after the end of cytotoxic therapy in the PROMISE-GIM6 study (from 25.9% to 8.9%; OR 0.28; $p < 0.001$) [71]. In the PROMISE-GIM6 study, the protective effect on ovarian function recovery was confirmed also at a longer follow-up with a 5-year cumulative incidence estimate of menstrual resumption of 72.6% in the LHRHa arm and 64.0% in the control arm (age-adjusted HR 1.48; $p = 0.006$) [70].

Moreover, more patients treated with LHRHa during chemotherapy had a subsequent pregnancy as compared to those undergoing chemotherapy alone: 22 vs. 12 (OR 2.45; $p = 0.03$) in the POEMS-SWOG S0230 study [69] and 8 vs. 3 in the control arm (age-adjusted HR 2.40; $p = 0.20$) [70]. Finally, both studies showed no negative impact of concurrent administration of LHRHa and chemotherapy on patients' prognosis [69, 70].

A large and updated meta-analysis evaluating the role of temporary ovarian suppression with LHRHa during chemotherapy in young women with breast cancer confirmed the potential efficacy of the procedure in preserving both ovarian function and fertility [72]. In the 12 randomized studies included in the analysis, for a total of 1231 patients, the use of LHRHa was associated with a significant reduced risk of developing chemotherapy-induced POF (OR 0.36; $p < 0.001$) [72]. In the five studies reporting number of patients achieving pregnancy, more women treated with LHRHa become pregnant after treatment (33 vs. 19; OR 1.83; $p = 0.041$) [72].

An individual patient data meta-analysis of randomized studies in breast cancer patients (the MOMMY study; PROSPERO registration number: CRD42014015638) is currently ongoing and is awaited to corroborate these findings [13].

According to the recently released BCY2 recommendations taking into account all the recent data on the topic, the expert Panel agreed that this strategy can be discussed with women interested in potentially preserving ovarian function and/or fertility [7]. Updated recommendations from ESMO and ASCO on this topic are warranted.

Conclusions

Fertility preservation and the possibility to have a family after cancer diagnosis and treatment have an important impact on quality of life of breast cancer survivors. The importance of preserving fertility at the time of diagnosis is also confirmed by the fact that pregnancy after breast cancer showed to be safe also in patients with hormone receptor-positive disease [24].

Fertility preservation strategies should be discussed with patients as soon as possible after breast cancer diagnosis [5–7] (Fig. 61.1).

For patients interested in fertility preservation (i.e., to become pregnant after treatment), embryo and oocyte cryopreservation are standard strategies and should be offered as first choice. The best candidates for these strategies are patients under the age of 38, with normal ovarian reserve at baseline and the possibility to delay the initiation of anticancer treatments for 2 weeks (Fig. 61.1). The use of "random-start" protocols and letrozole or tamoxifen for controlled ovarian stimulation should be considered in breast cancer patients [13]. In those patients who cannot delay anticancer treatment, cryopreservation of ovarian tissue can be offered (Fig. 61.1). In both scenarios, as well as for patients with no access to cryopreservation strategies, temporary ovarian suppression with LHRHa can be offered during chemotherapy to increase the chances of post-treatment recovery of ovarian function and fertility (Fig. 61.1).

For patients interested in ovarian function preservation (i.e., to avoid the negative consequences of treatment-induced POF) more than fertility preservation, temporary ovarian suppression with LHRHa during chemotherapy can be offered (Fig. 61.1).

Although several efforts in the field have been done in the last years, the lack of large prospective studies and randomized trials highlights the importance of further research. Registries and prospective studies are currently ongoing with the aim to better evaluate the efficacy and safety of the available strategies for fertility preservation. The participation to these studies should be encouraged to acquire more robust conclusions on these crucial issues.

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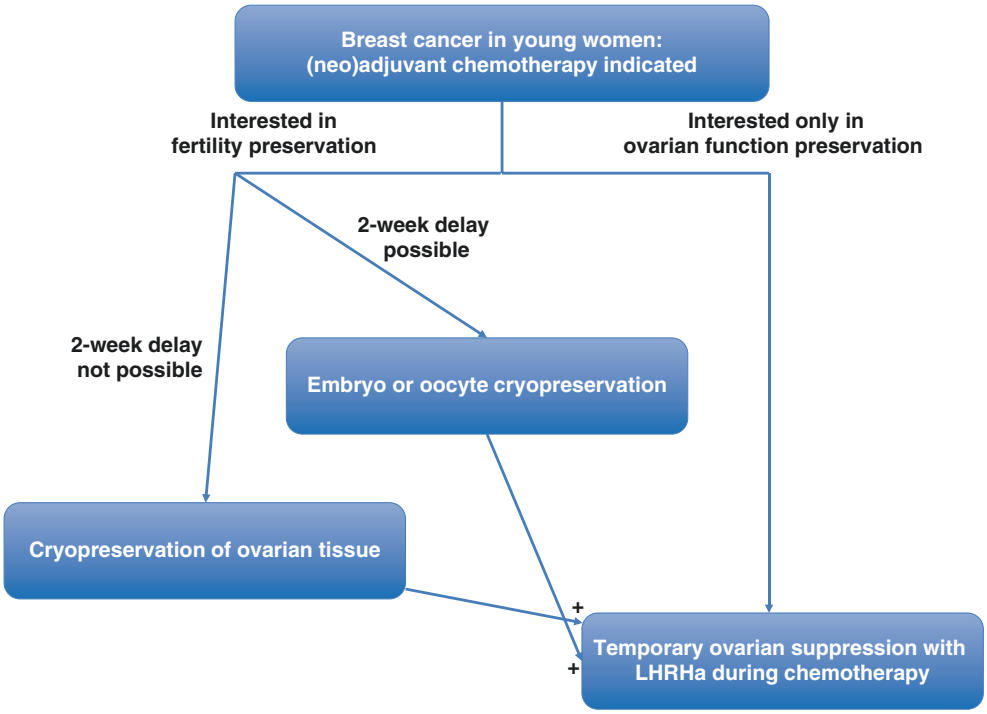

Fig. 61.1 Algorithm for young breast cancer patients candidates to (neo)adjuvant chemotherapy and interested in fertility and/or ovarian function preservation. Abbreviation: *LHRHa* luteinizing hormone-releasing hormone analogs

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Breast Cancer and Sexuality with Focus in Young Women: From Evidence-Based Data to Women's Wording to Treatment Perspectives

62

Alessandra Graziottin

62.1 Introduction

Sexuality is an integral part of quality of life. Young women affected by breast cancer (BC) face a very demanding disease, both physically and emotionally devastating [1–3]. They constitute a minority of BC patients (www.cancer.org): approximately 2% of breast cancers occur in young women between 20 and 34 years of age and 11% between 35 and 44 years of age [4], or 7% before the age of 40, but commonly have distinct concerns and issues compared with older women, including queries regarding sexual dysfunctions, fertility, contraception, pregnancy, premature menopause and couple intimacy. Young women' sexuality is threatened by BC in all its dimensions: sexual identity, sexual function and sexual relationship [2, 3].

The younger the woman, the less realized the different key goals of her life cycle (falling in love, having a satisfying sexual life, forming a stable couple, getting married, having a family), the more pervasive the consequences on her sexual identity, sexual function and sexual relationship can be [5, 6].

In 1–2 years after the diagnosis, a young woman may have to face three important losses: loss of physical integrity, as BC diagnosis and treatment may deeply affect her body image [3], her sense of health, physical energy, wellbeing, femininity and sexuality; loss of her possibility to become a mother and have her own children when iatrogenic menopause destroys the ovarian reserve [5, 6]; and loss of the couple relationship when progressive sexual dysfunctions disrupt the couple intimacy [2, 3, 5, 6] and/or when he abandons her as he cannot accept to remain childless because of her iatrogenic infertility.

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Psychological and psychosexual factors in young cancer survivors have been well studied [7–25], while biological factors contributing to sexual impairment are underestimated, underinvestigated and undertreated, with far less data [26–31], fortunately recently increasing [32–38]. Key issues of BC in young women are distilled in Table 62.1.

This paper will focus more on the biological side of the sexual concerns. As data specifically focused on younger women' sexuality are limited, the impact of BC on sexuality is (also) based on the evidence gathered from women of all ages, with notes on data gathered in the young. The impact of early iatrogenic menopause on sexuality will be carefully considered, analysed through the extensive clinical experience with young cancer survivors of the presenting author (AG). Attention to women's wording will be privileged, as individual experiences cannot be conveyed in controlled studies, and so many intimate issues can be transmitted by a single sentence. *Wording's reporting is focused on the experience of women who have more difficulties to cope with* the many challenges breast cancer carries with it, with the specific goal of increasing healthcare providers' awareness on issues usually marginalized in the oncological conversation with the patient. Moreover, this paper will focus on sexual concerns and touch fertility and pregnancy-related problems only in their specific impact on young women's sexuality. A concise paragraph on multimodal approach to sexual concerns and problems in young breast cancer survivors will conclude it.

Table 62.1 Key issues in young BC women, in comparison to older

BC is uncommon in young women, less than 2% under the age of 34 and 11% between 35 and 44
It is more aggressive, with later diagnoses and poorer outcomes
When breast-conserving therapy is performed, the rate of local recurrences is higher
Different key goals of a woman's life cycle may not yet be realized in younger women. Therefore issues on fertility preservation, pregnancy, sexuality, couple's erotic intimacy and premature menopause must be taken into the highest consideration and openly discussed with patients and, when appropriate, with couples

Modified from Axelrod et al. [32]

U. Veronesi et al. (eds.), *Breast Cancer*, DOI 10.1007/978-3-319-48848-6_62

62.2 Young Women's Wording on their Sexuality After Breast Cancer

To set the scenario, listening to women's words is essential. They well describe the overall sense of health and sexual loss they feel when early breast cancer diagnosis disrupts their lives. "Without my breast I do not feel a woman any more", "My breast is cold now, it does not belong to me anymore. It is like having a stone there". "I can't look at it myself, how can I show it to my partner?"; "I had breast cancer at 34 and underwent contralateral mastectomy at 37. None told me that I would have had chronic breast pain, besides losing all my sexual pleasure"; "I used to be a fighter. I went through surgery, radio and chemotherapy with lots of courage. My oncologist congratulated me at every visit. Since I started aromatase inhibitors, my insomnia went worst, I have night tachycardia, in the morning I get up exhausted, I have no more energy and no sex drive; but the worse of all is my joint pain. I feel trapped in an armour of sadness and rust. I do not recognize myself anymore"; and/or "Why should I make love, If I cannot have children anymore?": all these sentences concisely explain why sexual identity, the sense of femininity and sexual attractiveness and the potential for pregnancy are perceived as definitely wounded or lost.

"It's a disaster, doctor, my breast is frigid. And I'm becoming all frigid as well. I can't help"; "Since I lost my periods, after the chemotherapy, my sexual desire faded away. I do not have any sexual interest, for anybody. I feel sexually invisible"; "Since I became menopausal I have a worsening vaginal dryness; sex is no more fun. Now it hurts! And I have cystitis two-three days after the intercourse. But nobody cares. My oncologist keeps on saying: your life is more important. But which life is this, if I cannot make love anymore, at 32?!"; "I find any excuse to avoid sex with my partner: it only means pain": these sentences focus more on sexual function.

"We were looking for our first child. The breast cancer diagnosis shocked us deeply. At the beginning, my husband was very supportive. But when he realized I was getting menopausal because of chemo, he became more and more depressed. He was so longing for a child, and he said he cannot accept to remain childless. Please give me all the info on how I can get pregnant. Do I have a last chance with my ovary? Is ovodonation safe, after breast cancer? I do not want to lose him. We feel so lone and yet we cannot make love anymore...": this story focuses on sexual relationship.

These requests for a complex sexual help indicate how pervasive the discovery of a breast cancer and associated premature menopause can be for the three dimensions of young women's sexuality [2, 3, 5–33] and how we must keep in mind the complexity, to offer a well-tailored, individualized sexual help. A multidisciplinary approach, medical and psychosexual, may offer the most comprehensive and satisfying outcomes [2, 34, 37, 38].

62.3 Female Sexual Identity Issues in Young BC Women

Female sexual identity defines the satisfied sense of belonging to the female gender [2]. Such a comprehensive concept stems from basic dimensions such as femininity, maternity and eroticism, while social role can be considered a more recent contributor [2]. The perception of female sexual identity may be variably affected by BC diagnosis and treatment; the younger the woman, the more pervading the negative effect.

1. Femininity may suffer a major insult, for a number of biological reasons:
   (a) *Changes in appearance, dimensions, skin temperature and erotic feelings of the breast,* which is a prominent personal and social sign of femininity. *Body image* is one of the parameters considered to be more affected by the type of surgery performed. However, if women's wording is carefully listened to, the more recurrent word is "body feelings": "I cannot accept any caress or kiss on my breast: I feel nothing!". Short-term impact depends on the type of surgery performed: lumpectomy versus mastectomy, with immediate or delayed reconstruction, and their cosmetic result and the need or not of adjuvant radio- or chemotherapy and hormonotherapy [2, 3, 10–12, 15, 20, 25, 36]. Body image is not only visual, but is biologically based as well on proprioceptive, tactile and pleasure-related sensations [3]: "I underwent mastectomy and reconstruction. I was very proud with the cosmetic result. Then I realized that caresses, kisses, all was *fiction of sex without feelings*. I felt my breast has been killed twice. *It's a show without music*". Nipple sparing techniques may contribute to maintain a better body image, better feelings in the nipple, areola and breast and better sexual function. However specific studies focused on this aspect have not yet been carried out in the author's knowledge. Contralateral (CPM) and bilateral prophylactic mastectomy (BPM) adds further concerns to women' sense of femininity [3, 39, 40].
   (b) *Arm lymphedema* that causes a disfigured body image and impaired self-perception: "You can mask your breast surgery, but lymphedema reminds you and everybody around you that you had a breast cancer. I always have to wear long sleeves, like a

"chador". I can't even undress in front of my partner". Lymphedema wounds the inner sense of femininity, leading to depression and avoidant coping strategies [2, 3, 10, 11]. It has an average reported incidence of 15–30% [16, 29, 30], up to 42% in the more recent prospective research of Norman et al. [32]. It is the more serious side effects of axillary lymphadenectomy; however, it is reported in 1,8% of women who underwent the lymph node' sentinel biopsy. It may develop up to 20 years after breast and axillary surgery [30]. "Arm problems" are quoted by 43 to 72% of patients, according to the different arm symptoms (pain, pins and needles, numbness, skin sensitivity, swelling) that were mentioned in Ganz et al. [13], by 26 to 36% in Dorval et al. [10]. Unpleasant feelings ("paraesthesias") up to frank pain are frequently reported. Breast cancer is more advanced in younger women and requires more frequently mastectomy and lymphadenectomy [1, 33]: lymphedema and associated signs and symptoms can be significant (and underappreciated) contributors of the femininity crisis in the youngest BC cohort.

(c) *Iatrogenic menopause* is the third biological factor that may wound the sense of femininity of *younger patients* (25% of breast cancer patients are premenopausal, and 13% are diagnosed before the age 45) [1, 4]. Polyagent adjuvant chemotherapy may cause early menopause in 56 to 89% of BC women [41]: "It kills your femininity because getting menopause so young makes you feel suddenly old and lost for the love play". Things can be worse for the sense of femininity when chemotherapy causes both hair loss and premature ovarian failure (POF) or insufficiency (POI) [5, 6]. "I had beautiful, long black hair with blue nuances. It was my pride. During chemotherapy, I had a massive loss. My hairdresser was moved to tears when he had to cut them short. My whole femininity has gone with my hair". Loss of oestrogens triggers neurovegetative, affective and cognitive symptoms, increases vulnerability to depression, low sexual desire, vaginal dryness and dyspareunia and determines an accelerated ageing of sexual organs [2–6]. The disrupting role of premature iatrogenic menopause on sexuality has been extensively reviewed in previous papers [2, 3, 5, 6], with new contributions on young BC women [34–37]. Tamoxifen and aromatase inhibitors may further worsen the impact of iatrogenic menopause on the wellbeing and femininity [2, 3].

(d) *Age* is the fourth biological factor that may worsen the impact of BC on female sexual identity. Its specific weight is related to:

- Biological factors: BC in younger women has a more aggressive course and poor prognosis and requires more aggressive treatments (local recurrences are higher in younger patients) [1, 32], with a higher biological price in terms of short- and long-term side effects of treatments and comorbidities [41] and higher risk of a systemic disease at diagnosis [1, 32].
- Psychosocial factors: key social tasks and goals of women's reproductive years have different priority in different decades: the younger the woman, the higher the probability that key goals will not be achieved: "At 30, I'm alone. I lost my periods and my desire. I do not have a boy-friend, I feel I'm invisible. Sexually, I mean. I do not have children. The only thing I do have is a cancer that destroyed all my hopes of a worthy life". Problems associated with breast cancer continue to persist several years after diagnosis, and even worsen, at least for a consistent percentage of young women [24, 25], who should therefore be considered at higher risk of negative QOL outcomes after breast cancer and be offered a specific help [2, 3, 5–8, 14, 16–18, 25, 36].

2. Maternity: in young breast cancer patients who were childless at the moment of the diagnosis, it may become the core of a major sexual identity crisis [2] and trigger a critical fracture in the relationship. As the mean age at first pregnancy has rapidly increased in recent decades in high-income countries, the possibility that a woman has a cancer still being childless is growing: "The oncologist started the chemotherapy without mentioning that it would destroy my ovaries. Had I been informed, I could at least have saved my oocytes. Cancer is serious, but destroying my fertility is much more unbearable for me". Appropriate counselling on the POF risk and possibility of cryoconservation of oocytes before chemotherapy should be mandatory.
3. *Eroticism*: BC may affect sensuality, sexiness and receptiveness through:
   (a) The major insult of breast surgery on breast eroticism: 44% of women with partial mastectomy and 83% of those with breast reconstruction ($p < 0.001$) report that pleasure with breast caresses and sexual feelings have decreased [20], because of the local nervous damage. Surgery for breast cancer leaves women sore and tender in the breast and chest area on an immediate basis. Likewise, radiation therapy can cause skin to become red, tender and irritated. While pain and irritation might subside as the wound heals, either the woman or partner may avoid sexual interaction during this time period. This "collusion of avoidance" may dramatically impair the sexual importance of the breast [2, 3]. The dramatic loss of

Table 62.2 Negative changes in sexual enjoyment after BPM

Effect	Odds ratio
Lost/very impaired sexual sensitivity in the breast after BPM	8.631
Pain experiences in the breast after BPM	2.604
Discomfort feelings in the breast after BPM	3.887
The breast had great sexual importance before BPM	25.704

Adapted from Gahm et al. [40]

the "sexual meaning" of the breast has been demonstrated by the work of Gahm et al. [40] to be very significant also in women undergoing BPM, with an OR = 25.70 (Table 62.2);

(b) The menopause. Symptoms (hot flushes, sweating, night tachycardia, mood swings, insomnia, depression, joint pain, loss of desire, arousal difficulties, orgasmic difficulties, dyspareunia) [2, 3, 5, 6, 36], signs: wrinkles, weight gain [36], modified body shape ("the menopausal look"), swollen and painful joints, reduced muscle mass and strength ("sarcopenia"), mouth dryness, vaginal dryness [2, 3], vulvar dystrophy and quality of life impairment secondary to iatrogenic (chemotherapeutic) and/or nonhormonally treatable natural menopause may dramatically devastate the woman's sense of eroticism [2, 3, 20], besides the impact on her femininity. Moreover, these symptoms threat the core of the erotic perception a woman may have: "I loved to have sex, I used to be considered very sexy. Breast cancer shocked me, but the worse was the unexpected menopause that killed my sense of being a sexy woman. And the hormonal treatment I have to do makes me feel even worse. At 36, I cannot accept a life without sex, I'd rather die";

(c) The worsening impact of depression and anxiety, reactive to BC, that may affect self-perception, sense of sexiness and eroticism via nonhormonal pathways. It is reported in average 17 to 25% of breast cancer patients [37]. "When the immune system subjugates the brain" [42]: the inflammatory nature of the biological component of depression [43, 44] may explain why cancer patients are significantly more depressed than non-oncologic patients and why depressed cancer patients die more than nondepressed cancer controls.

4. Social role may represent an area relatively safe from BC, particularly in well-educated women [2, 45], except in the acute phase or in the more severe and aggressive cases. A strong and positive social role and a gratifying professional career may reduce the impact of BC on other dimensions of femininity [2, 13]. However, 20% of BC survivors report a reduction of energy, psychological distress, cognitive problems and difficulties in concentrating, in remembering and in thinking clearly that may affect their professional competence and role [13]. Cognitive deficits after post-operative adjuvant chemotherapy for BC have been described in a broad domain of functioning, including attention, mental flexibility, speed of information processing, visual memory and motor function [46]. This cognitive impairment is unaffected by anxiety, depression, fatigue, and time since treatment [46]. It may be more disturbing in women who rely on their professional and social role for their sense of identity and self-esteem.

62.4 Female Sexual Identity Issues After Bilateral or Contralateral Prophylactic Mastectomy

9 to 10% of women with breast cancer may have a genetic predisposition [1, 40]. The majority of these (55–70%) are caused by mutations of either *BRCA1* or *BRCA2* tumour suppressor genes and are associated with an increased risk of ovarian cancer. Bilateral prophylactic mastectomy (BPM), a preventive option in women carriers of these mutations, may specifically affect women's sexual identity and body image.

A Cochrane review focused specifically on outcomes of women undergoing BPM or contralateral prophylactic mastectomy (CPM) after breast cancer diagnosis [39]. Twenty-three studies, including more than 4000 patients, met inclusion criteria. All studies had methodological limitations. Most reported *high levels of satisfaction with the decision to have BPM* but more variable satisfaction with cosmetic results. *More women were dissatisfied* than satisfied *with the support they received in the healthcare setting*, which parallels the dissatisfaction on this domain after breast cancer diagnosis. Worry over breast cancer was significantly reduced after BPM when compared both to baseline worry levels and to the groups who opted for surveillance rather than BPM. Three studies reported *body image/feelings of femininity outcomes*, and all reported that a *substantial minority (about 20%) reported BPM had adverse effects* on those domains. Two case series were exclusively focused on adverse events from prophylactic mastectomy with reconstruction, and both reported rates of *unanticipated reoperations from 30% to 49%*. Of the psychosocial outcomes measured, *body image and feelings of femininity* were the most adversely affected [39]. Cochrane reviewers conclude that while published observational studies demonstrated that BPM was effective in reducing both the incidence of, and death from, breast cancer, more rigorous prospective studies (ideally randomized trials) are needed.

Gahm et al. [40] quoted that 85% of women reported reduced sexual sensations after BPM. Loss of breast's sexual meaning and even pain after BPM should be discussed

before surgery as they are even more relevant in the decision-making process when a preventive intervention is considered. On the surgeons' side, attention to the most skilled nerve preserving BPM, to maintain erotic sensitivity, with special attention to intercostobrachial nerve to prevent pain, is of the highest importance to minimize long-term negative sexual outcomes [47].

Recent studies on psychosocial effects of CPM confirmed the same data, even when a long follow-up period is considered (mean 10.3 years) [15, 48]. Decreased satisfaction with CPM was associated with decreased satisfaction with appearance, complications with reconstruction, reconstruction after CPM and increased level of stress in life [48]. Counselling on BPM and CPM should (also) discuss psychosexual and body image-related issues [40].

In summary Women's sexual identity in young women with breast cancer may be variably affected according to a number of biological factors: age at diagnosis; stage and type of treatment; type and cosmetic outcome of surgery, including the nipple sparing or not; presence and severity of lymphedema; accomplishment or not of childbearing before diagnosis; infertility; induction of POF leading to menopause with its cohort of symptoms and signs; severity of depression; and biological mental damage from chemotherapy and inflammation, further triggered by BC treatment and chronic loss of oestrogens and testosterone. The preventive meaning of BPM and CPM conveys specific issues for female sexual identity. The differentiation of the relative weight of these factors with respect to psychosocial variables deserves further prospective studies.

62.5 Female Sexual Function and Dysfunction in Young BC Women

Human sexual function can be simplified as a circuit, with four main stations: sexual desire and central/mental arousal, peripheral arousal with genital congestion and lubrication, orgasm and satisfaction that includes both the physical phase of resolution, with its homeostatic function of returning to baseline, and the emotional/affective evaluation of the experience [2] (Fig. 62.1). Sexual dysfunctions may anticipate breast cancer, be concomitant to it or consequent to treatment [2, 3]. They can as well be worsened by partner's attitudes [49]. Young BC survivors had significant poorer sexual functioning in every area of sexual functioning than normal control groups [26, 31, 33–36, 47, 48]. Interdependence of different aspects of sexual functions [50] translates in a complex vulnerability in all sexual function domains, more so in young women challenged on so many fronts of their life [51–53].

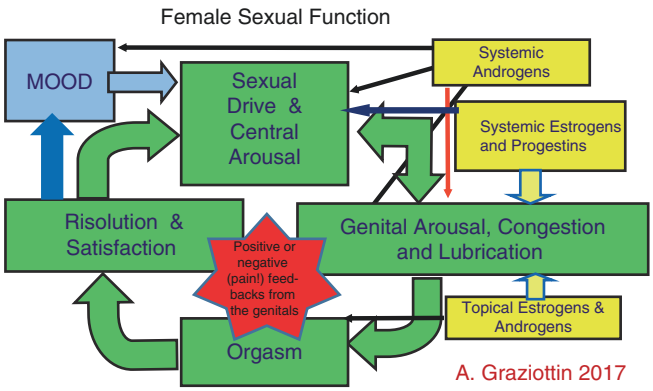

Fig. 62.1 This model, formulated by the author, indicates: (a) the interdependence of different biological dimensions of women's sexual function, desire and central arousal, genital arousal and lubrication, orgasm, resolution and satisfaction; (b) the possibility of positive or negative feedbacks, starting from any of the sexual dimensions; (c) the role of systemic sexual hormones in modulating the biological basis of sexual response in the brain, in the body and in the genitals and their key role in mood modulation; and (d) the role of topical/genital hormones in potentially improving the biological basis of vaginal lubrication, genital congestion and orgasm

Young women who received *chemotherapy*, and underwent POF, tended to desire less frequently ($p < 0.032$), had more vaginal dryness ($p < 0.001$) and dyspareunia ($p < 0.001$) and had sex less frequently ($p < 0.013$); the ability to reach orgasm through intercourse tended to be reduced ($p < 0.043$), although their ability to reach orgasm through noncoital caressing did not differ from that of other women [20]. Coital receptiveness is therefore selectively damaged [20]. This vulnerability depends on the effect of loss on oestrogens on vaginal lubrication and trophism [54]. The progressive thinning of the vaginal mucosa, vaginal dryness and dyspareunia, vulnerability to coital microabrasion, increase of the vaginal pH and altered vaginal ecosystem with increased vulnerability to infection and vaginitis from colonic germs, consequent to the early menopause, all reduce the possibility of enjoying intercourse [54]. Overall sexual satisfaction was significantly poorer ($p < 0.001$) [20]. In particular, Speer comparing BC survivors (age range 41–69 years, average time since diagnosis 4,4 years) vs. women affected by FSD and normal women obtained similar FSFI score (female sexual functioning index) between the first two subgroups vs. normal control group (<.001) [26]. Biglia et al. [36] confirm that young BC patients report significant reduction in frequency of intercourse and reduction in the mean score related to sexuality domain that worsens after 1 year of follow-up. Interestingly, only romantic/implicit stimuli significantly increased in power of evoking sexual interest after 6 months from surgery.

Young women who receive *hormonotherapy* may have a further sexual burden [31, 55–57]. Oestrogen receptor-positive BC women have been treated with *tamoxifen* (TAM)

for almost four decades. Although reasonably well tolerated, this worldwide used drug may specifically affect sexual function [31, 55–57]. The research of Merits et al. indicates that during tamoxifen therapy the most frequent complaints were hot flushes (85%), disturbed sleep (55%), vaginal dryness and/or dyspareunia (47%), decreased sexual desire (44%) and muscular-skeletal symptoms (43%) [55]. Disturbed sleep correlated with hot flushes ($p < 0.0005$) and concentration problems ($p < 0.05$). Decreased sexual interest correlated with vaginal dryness ($p < 0.0005$) and/or dyspareunia ($p < 0.0005$). After discontinuation of tamoxifen, symptoms decreased significantly [55]. Moreover it has been demonstrated that women taking TAM have more difficulties achieving orgasm than women who are not taking this drug [57].

Treatments with *aromatase inhibitors* (AI), such as anastrozole, letrozole or exemestane which inhibit the conversion of androgens to oestrogens, have a negative impact on sexual response in oestrogen-depleted women, as they may exacerbate menopausal symptoms and sexual sequelae [55–57]. Morales et al. [56] published the results of a prospective study including 181 postmenopausal BC survivors scheduled to start endocrine treatment. A menopause questionnaire had been fulfilled at the baseline, after 3 and 6 months of therapy. Both first-line TAM and AI induced an increase in the occurrence and severity of hot flushes ($p < .0001$ and $p = .014$, respectively). Musculoskeletal pain and dyspareunia significantly increased under AI ($p = .0039$ and $p = .001$, respectively). Sexual desire was reduced only under TAM treatment ($p < .0001$). Younger patients suffered more of hot flushes and/or vaginal dryness ($p = .02$) [56].

Different dimensions of sexual function are interconnected. In parallel, interdependence exists among sexual dysfunctions. Research data indicate that comorbidity among sexual dysfunctions is prominent also among young BC patients [2, 3, 14–16, 19, 20, 22, 26, 31, 35, 36, 39, 40, 51–53, 55–57]. However, as specific pathophysiologic vulnerabilities exist in each sexual domain, a concise analysis of each sexual dysfunction in young BC women will be reported.

62.6 Sexual Desire Disorders in Young BC Patients

Sexual desire encompasses **biological**, **motivational-affective** and **cognitive** contributors [50].

1. *Biological roots* of sexual desire depend first on sexual hormones, which are necessary but not sufficient factors to maintain a satisfying human sexual desire [2, 5, 6]. *Hormones* seem to control the *intensity* of libido and sexual behaviour, rather than its direction [5]. Loss of desire up to a frank hypoactive sexual desire disorder (HSDD) is the most frequent sexual complaint in young BC patients, reaching 64% of BC survivors [51]: vulnerability increases after iatrogenic premature menopause [28, 35, 36, 51–53] and if hormonal treatment are used [31, 32, 36]. After BC treatment, loss of oestrogens, secondary to iatrogenic or naturally occurring menopause, may contribute to inhibit the sexual drive and the physical receptiveness; loss of androgens [35], secondary to chemotherapy or ovariectomy, may further worsen the picture. *Depression* is currently understood as a systemic disease with a major inflammatory basis [42–44]. It is the psychiatric disorder more frequently associated with loss of desire in women and in men and is significantly increased in young BC patients. A lower mood level in young BC patients was detected in 63.6% of the study population, reporting irritability, retardation in daily activities and episodes of crying [36]. A reduced desire score, evaluated by FSFI scale, among BC vs. normal women ($p < .001$) has been reported [26]. Loss of sexual desire is a multifactorial problem that may be as well *secondary to* arousal disorders, with vaginal dryness and dyspareunia, orgasmic disorders, sexual dissatisfaction and relationship issues [2–6, 31].
2. *Motivational-affective and cognitive aspects* of sexual desire may be impaired by the negative impact breast surgery has on body image, femininity, eroticism, seductive confidence a woman feels and overall quality of life [2, 3, 51–56]. The shift of couple relationship towards more affective dynamics may increase emotional intimacy but reduce the physical component of sexual drive in both partners [2, 3, 45, 49].

62.7 Sexual Arousal Disorders, Vaginal Dryness and Dyspareunia in Young BC Women

Sexual arousal indicates a mental and physical state with specific feelings, usually attached to the genitals [54]. Breast cancer survivors may suffer from complex arousal disorders, secondary to:

1. *Biological central/mental difficulties* caused by the loss of sexual hormones, secondary to iatrogenic or spontaneous menopause, which may be made worse by depression, anxiety, chronic stress and insomnia [54], triggered by the cancer diagnosis and worsened by oestrogen loss and/or hormonal treatments [2, 51–53, 55–57]. Reduced frequency of erotic dreams, of fantasies, of sexual day dreams and of spontaneous mental arousal are the clinical

consequences of central arousal difficulties that can be reported to the listening physician in young BC patients: "The first thing that made me feel in love were erotic dreams; now nothing thrills my fantasies again" or "I'm so sexually indifferent now that it takes ages of foreplay to be turned on".

2. *Problems in non-genital peripheral arousal* may be better exemplified by "touch-impaired" disorders: nipple erection and feelings may be reduced by decreased breast sensitivity, secondary to surgery: this is a most frequent (and neglected) complaint after BC surgery [3, 40]. Nipple and breast loss of arousability can be worsened by the shame some women feel in exposing the operated breast and having it touched or caressed by the partner [3].
3. *Inadequate genital arousal, reducing: a) vaginal lubrication, causing vaginal dryness, and b) cavernosal bodies congestion, leading to reduced/absent clitoral and cavernosal bodies arousal.* Oestrogens have a prominent "permitting" role on the action of vasoactive intestinal peptide (VIP), the most important neurotransmitter that "translates" sexual drive into vaginal lubrication, while testosterone is the permitting factor for nitric oxide (NO) that mediates most of the cavernosal body congestion [54]. Without oestrogens, *vaginal dryness* and *dyspareunia* are complained of by 47 [55] to 61% [13] of young breast cancer survivors: "I feel dry in spite of a very loving and careful partner. My vagina has forgotten what being wet was. And it hurts most of the time". Pre-existing arousal disorders may be further worsened by the menopausal loss of oestrogens and loss of libido many women complain of after breast cancer [2, 3]. Loss of testosterone, caused by the iatrogenic menopause [35] that significantly reduces the ovarian production by the Leydig cells, explains the increase in the time needed to get a pre-orgasmic clitoral congestion—"It takes ages to get aroused"—and consequent orgasmic difficulties. Another biologic cause of arousal difficulties is the *defensive spasm of pubococcygeus muscle*, either primary, in patients who were suffering from vaginismus and lifelong dyspareunia, or secondary to vaginal dryness and coital pain [2, 3, 58] that further narrows the entrance of the vagina, worsening the vestibular pain until it may contribute to vulvar vestibulitis/vulvodynia: "After the chemotherapy was completed, I started having pain at the entrance of the vagina because I'm dry and tight. And after the intercourse, I have the feeling of many little cuttings in the vagina that burns for two-three days. It's becoming a nightmare. The worst is that I consulted three gynaecologists and they say I have nothing and it must be a psychological problem. But I'm inventing nothing! It really hurts!" [58].

Overall, in the prospective longitudinal study of Ganz and co-workers [13], difficulty in becoming sexually aroused was reported by 61% of BC patients, while difficulty in getting lubricated was found in 57% of the patients. Interestingly, Ganz et al. [13] found that BC survivors attain maximum recovery from the physical and psychological trauma of cancer treatment by *1 year* after surgery. A number of aspects of QOL, rehabilitation problems (mostly *arm problems*) and *sexuality* significantly *worsen* after that time, suggesting that some biological factors might be responsible for this unfavourable trend. According to the retrospective study of Schover et al. [19], BC women who received chemotherapy reported more vaginal dryness ($P < 0.001$) and dyspareunia ($P < 0.001$). Overall, postmenopausal BC women (both for natural or iatrogenic early menopause) were more likely to report vaginal dryness and tightness with sexual activity ($P < 0.001$) and genital pain with sexual activity ($P = 0.004$). The role of the possible *worsening over time* of the biological basis of the sexual response deserves to be tested in new prospective studies, analyzing biological factors from a stringent pathophysiologic point of view.

62.8 Orgasmic Disorders in Young BC Patients

Orgasmic difficulties may be the end point of a number of biological, as well as motivational-affective, cognitive and behavioural factors, such as the quality of the foreplay, affecting desire, central and peripheral arousal, with reduced genital vascular, nervous and muscular response. In BC patients difficulty in reaching orgasm is reported in 55% of patients in the prospective longitudinal study of Ganz et al. [13], with a significant worsening in sexual functioning over the 3 years of follow-up. A very frequent finding in young BC patients after an iatrogenic menopause is the following: "I had an early menopause at 35, after chemo. Now, at 39, it takes ages to get aroused, my orgasm is difficult to reach and very weak. I'm more and more frustrated".

In the Schover et al. retrospective study [20], the ability to reach orgasm through intercourse tended to be significantly reduced in women who received chemotherapy ($P = 0.043$) although their ability to reach orgasm through noncoital caressing did not differ from control women. Inhibitory effect of dyspareunia on vaginal orgasm might explain this difference. FSFI score about orgasm was higher in BC survivor patients vs. FSD women, but lower vs. controls ($p < .001$) [26]. Orgasmic dysfunction ranges from 16–36% of young BC women in more recent studies [51–53]. 41% of women on tamoxifen treatment report orgasmic difficulties in Merits' et al. study [55].

62.9 Sexual Dissatisfaction in Young BC Patients

Sexual satisfaction is a comprehensive and yet elusive word [2]. It includes both physical and emotional satisfaction that should probably be investigated as separate parameters. Pain and an overall disappointing sexual experience might also be responsible for the significantly reduced satisfaction ($P < 0.001$) reported by BC survivors in the retrospective study of Schover et al. [20] and in the prospective study of Dorval et al. [11] who as well report a significantly reduced satisfaction ($P < 0.003$) in BC survivors, 8 years after primary treatment, in comparison to age-matched controls. "I force myself to have sex with my husband not to lose him, but I have no satisfaction at all. It's the last homework before sleeping. I'm so frustrated…". Recently, scoring satisfaction with FSFI, Speer showed a reduced average satisfaction among BC satisfaction vs. controls and vs. non-cancer FSD women ($p < .001$ and $p < .05$, respectively) [26].

In Summary Breast cancer in young women affects almost every domain of **sexual function**, causing loss of sexual desire, arousal difficulties with vaginal dryness and dyspareunia, orgasmic difficulties and dissatisfaction in a significant proportion of patients; the younger the woman, the higher the vulnerability. Biological factors dramatically impair the pathophysiology of sexual response, while psychosocial issues may further contribute.

62.10 Sexual Relationship in Young BC Women

Quality of affective bonds, and specifically of sexual relationships, both homo- and heterosexual, is a critical part of human wellbeing and adult satisfaction. A good quality of emotional intimacy may explain why 62% of BC patients found it easier to discuss their sexual problems with their partner during their illness than with doctors and psychologists, to whom only 15% of BC patients dared to openly express their concerns [14].

Cancer diagnosis is a tremendous strain factor on the couple relationship and on the family [3, 18, 49, 59]. Young women and couples are particularly vulnerable as sexuality is a prominent need in young human beings, deeply motivated by the pursuit of physical pleasure and passion. "Sex was the best part of our marriage. After my breast cancer, I could not have sex anymore because of pain at intercourse, and my dry vagina. I'm so worried that my husband will leave me because of that…". Frustration of sexual needs is therefore more difficult to cope with and to be accepted in young subjects and couples in comparison to older ones.

Studies indicate that younger women experience more emotional distress than older women [18, 59]: "Since I started the aromatase inhibitors, the vagina became more and more dry. I want to have sex in the dark, so that my husband will not see tears pain at intercourse causes me... Why do I want to have sex if it hurts so much? You know, I have a metastatic disease. Making love together makes me feel so deeply connected. I need it now more than ever". Younger husbands reported more problems carrying out domestic roles ($P < 0.001$) and more vulnerability to the number of life stressors they were experiencing ($p < 0.01$) in comparison to older husbands. "At the beginning my husband was very supportive with me and the children and seems to cope well with the nightmare my cancer caused to all of us. But I was devastated when I discovered he had an affair with a beautiful and healthy woman…". When BC is diagnosed the demands of illness are superimposed on the normal routine of family life, and this may have a different impact on the family relationships depending on the phase of the family life cycle when the cancer is diagnosed [18].

Focusing on the physical aspect of the problem, breast surgery may affect physical attractiveness and reduce easiness with breast foreplay in the partner, although this is difficult to be openly admitted as it seems rough, insensitive and/or unfeeling [3]: "Since I got breast surgery, my husband didn't want me anymore", she says, while he says: "I did not dare to make any sexual advance to my wife, as I felt she was so shocked by the cancer diagnosis, and treatment was so hard on her…". Lack of communication may create a kind of "glass wall" between the partners that hurts both of them.

Loss of oestrogen may also make penetration more difficult because of vaginal dryness [2, 5, 6]. It may precipitate an erectile deficit, when dryness itself challenges the quality of the erection or when the partner perceives vaginal dryness as a sign of refusal or somehow an indication of the "insensitivity" of his sexual request and approach [2, 5, 6]. It may impair male physical and emotional satisfaction, when the instinctual drive is braked by physical difficulties and emotional concerns. The concept of "symptom inducer" and "symptom carrier", experienced in many sexually dysfunctional couples, may explain why addressing sexual issues with couples may be more effective than working with the individual BC woman [2, 34, 60].

In Summary Sexual relationships in young BC women can be more affected than in the older group because of the severity of the biological impairment of the sexual response, due to the sudden negative consequences of iatrogenic menopause and hormonal treatment; the stronger meaning sexual pleasure has in young people; the heavier burden of routine tasks in young families; and lack of communication between partners.

62.11 Treatment Options in Young BC Women with Sexual Concerns

The majority of young BC women complain that their sexual problems remain unaddressed, more so from the physical point of view: "I'm fed up of talking about feelings and mourning my losses with my psychologist. I need someone very practical that helps me to get rid of all my vagina pain and have a decent sex back!".

Recommendations to ease the difficult sexual life of young BC patients are based most on clinical experience of the author; evidence-based data will be quoted when available.

1. Lifestyle intervention:
   (a) *Daily exercise*—1 h crispy walking, gym, swimming, jogging, yoga, etc. may improve:
   (a) body shape and body weight, general mental tonus, assertiveness and self-confidence, contributing to a better body image and better sexuality [61]; (b) mood, sleep, appetitive-seeking-lust system and endorphins, reducing irritability, tension and aggressiveness [62]; (c) It reduces the burden of negative emotions associated with BC diagnosis and treatment; (d) Experimental data show that 20 min of jogging before sexual stimulation significantly improves genital arousal in normal women and women taking antidepressants [63]; (e) Breast cancer risk of recurrence is significantly reduced by regular exercise, possibly (also) through a reduction of systemic inflammation; (f) Lymphedema risk is reduced, while body image, body feelings and sexuality are improved by reducing body weight and by daily exercise [64];
   (b) *Appropriate diet*, with preference for legumes, cereals, fruits and vegetables, and no alcohol, to contrast the tendency to increase body weight after the earlier menopause [36], synergize with physical exercise in maintaining a younger, more gratifying body shape and body image [61], reducing neuroinflammation and systemic inflammation and reducing the risk of recurrences.
   (c) *No smoking* should be recommended, as it has specific negative effects on genital arousal (besides its oncogenic potential) in women, as it is well known in men, and specifically reduces genital congestion and vaginal lubrication [65], contributing to vaginal dryness and dyspareunia.
   (d) *Breast self-massage*: (a) to improve the quality of scarring, when appropriate, with medicated cream/oil, and reduce negative breast/arm sensations/feelings through the nervous "gate control" system; (b) to promote at least a partial "neuro-rehabilitation" of nipple and breast sexual feelings through the recall of former pleasurable sensations during the massage itself; and (c) to (re)integrate the breast in the body image, body feeling and body love map.
   (e) *Vaginal self-stretching and self-massage*, to relax the hyperactive pelvic floor, reduce pain associated with levator ani myalgia (underdiagnosed and undertreated in BC women) [58] and ease penetration. Gentle stretching and massage can be taught to a caring partner and included in the foreplay [58].
   (f) *Resume sexual intercourse soon after BC surgery*, if desired, with appropriate suggestions to ease it. There is no medical contraindication to sexual activity in BC patients, but it should be clearly mentioned to the couple by the consulting physician. Moreover, it has been found that regular sexual activity decreases symptoms of atrophy, both in medical examination and patients reporting, increases sensation and improves sexual satisfaction in normal women [66]. Research is needed in BC patients.
2. Physiotherapy:
   (a) To prevent and reduce *lymphedema* [64], when indicated
   (b) To relax the *pelvic floor* and reduce the hyperactivity associated with coital pain at the entrance of the vagina ("introital dyspareunia"), contributing to vestibular pain/vulvodynia and/or post-coital cystitis [58, 67]
3. Pharmacological interventions:
   a. NON hormonal drugs:
      - *Systemic*
        – *To reduce:*
        Hot flushes: gabapentin, low-dose SSRI, with attention to possible interactions with cytochrome P450 [34]
        Insomnia: melatonin
        Depression: SSRI at low effective doses, to minimize the potential negative effect on sexual function
        – *To improve sexual function:*
        Bupropion has been shown to increase sexual desire, arousal, orgasm intensity and overall sexual satisfaction in healthy women, and it may be considered for BC women; studies are needed [68]
        Phosphodiesterase type 5 (PDE 5) inhibitors (tadalafil, sildenafil, vardenafil) to improve genital arousal and lubrication, used despite the lack of FDA approval [34]
        Ospemiphene, a selective oestrogen receptor modulator (SERM) to be used after completion of adjuvant therapies
      - Topical (vaginal) nonhormonal:
        hyaluronic acid
        colostrum gel

Evidence support their efficacy in non-BC women complaining of vaginal dryness. They are intrinsically safe for

BC patients, as they do not contain oestrogens nor other sexual hormones, although specific studies on the effectiveness in BC patients deserve prospective studies.

- Topical (genital) hormonal drugs to improve:
  - Vaginal/bladder symptoms:
    Vaginal promestriene (the weakest synthetic oestrogen with less than 1% absorption), where available.
    Vaginal estriol and estradiol to relieve vaginal atrophy/dryness, post-coital cystitis and dyspareunia [58, 69, 70] to be decided in individual cases when they remain unaddressed with conventional non-pharmacologic treatments such as lubricants. Ospemiphene might offer a new treatment opportunity, oncologically impeccable at the end of the adjuvant treatment.

Oestrogens remain controversial and contraindicated in BC patients. However many physicians advocate a tempered approach aiming at tailoring the use of minimally absorbed local vaginal oestrogens, at least in those women complaining of a critical and unacceptable worsening of their intimate life. Patients should be appropriately counselled and consented and the conversation carefully documented in the medical record [34].

  - Vulvar symptoms of dryness and low arousability:
    Topical testosterone, propionate 1 or 2% cream in Vaseline or vitamin E gel, or testosterone of vegetal derivative in Pentravan (galenic preparation) is used off-label: same concerns and cautions as for vaginal oestrogens must be considered; preliminary studies on topical testosterone gel are ongoing [71], and new data suggesting the antiproliferative role of testosterone are raising new hopes on the possibility of safely using it to improve genital and sexual symptoms.
    Topical dehydroepiandrosterone (DHEA) cream (Labrie data) is the precursor of oestrogens, testosterone and progesterone. It interacts with oestrogen receptor beta, with antiproliferative and reparative actions. At the doses studied by Labrie and co-workers, it has only vaginal activity with no systemic absorption. Data in normal women treated with DHEA, in vaginal ovules, suggest efficacy with vaginal atrophy and no statistically significant changes in hormonal level [72]; it could therefore be considered in BC patients to improve vaginal lubrication and genital arousal. However, prospective data are needed in BC patients to confirm efficacy and safety.

4. Non-pharmacological methods:
   (a) The *Eros clitoral therapy device*, a clitoral vacuum pump approved by the FDA, can improve clitoral congestion [34].
   (b) *Vibrators* can be helpful for extra stimulation of vagina and clitoris in BC women. However both methods may be difficult to be accepted by many patients. Besides, loss of oestrogens and testosterone, and the parallel involution of the cavernosal bodies, reduces progressively the anatomic basis (and potential) of an appropriate genital congestion, even with stronger mechanical stimulation.
   (c) *Lubricants*: water-based and silicone lubricants can be used, as they do not reduce condom efficacy; over-the-counter products such as warming agents, bactericides, spermicides, perfumes and artificial flavours should be avoided as they may irritate the vaginal mucosa and worsen local symptoms and pain [34]. However, some younger patients and partner object that lubricants are "a humiliating fiction of arousal". That's why treatments that enhance the physiologic genital arousal response would be very welcomed, particularly in the younger BC cohort.
5. Individual intervention:
   (a) *Mindfulness-based interventions*: this kind of meditation reduces fear of recurrence, decreases stress, anxiety and depression and has been proven useful in BC patients [73]. It also improves disturbed sleep, promotes higher energy, combats fatigue and increases calm and wellbeing in a non-cancer population, potentially improving sexual function as well. Research is needed in young BC patient;
   (b) *Psychoeducational intervention*: this 6 weeks trial improved relationship adjustment and communication and increased sexual satisfaction [60].
6. Couple intervention:

Relationship enhancement intervention: a six-session biweekly trial, aimed at improving emotional expressiveness, problem-solving skills and self-growth, was performed in BC patients. It improved sexual functioning [74]. The background of this approach is that the women's view of her body is heavily influenced by her partner's response to her body. Its efficacy stresses the importance of couple-based approaches [74]. Controlled studies suggest that interventions focused on the couples rather than on the individual woman have the most likelihood of producing statistical and clinical improvement. The clinical experience supports this finding: it's crucial to give more balanced help also to partners of BC survivors [2]. All the more as husbands and couples express their relief and gratefulness when these issues, potential difficulties and/or misunderstandings are openly and spontaneously raised by the physician during the consultation and when practical suggestions are given to overcome physical and emotional sexual problems.

7. Partner support:

Medical and/or psychosexual support may be indicated for his or her partner, when needed.

62.12 Future Treatment Options for Young BC Women

1. Hormonal:
   (a) *Estetrol (E4)* systemic or vaginal: this weak foetal oestrogen has antioestrogenic effect on the breast and very promising oestrogenic effects on the brain, bone, joints and vagina. If safety and efficacy data will be confirmed, E4 could be the drug of the future to help BC patients of all ages, as it may reduce/eliminate all the menopausal symptoms while maintaining the antioestrogenic protective effect on the breast [75]. Studies are ongoing.
   (b) *Testosterone, systemic*: it still evokes discussion in BC patients because of the possible aromatization of testosterone to oestrogens. More research is needed in terms of systemic testosterone and its safety in BC patients [76].

62.13 Conclusions

Breast cancer may affect young women's sexual identity, sexual function and sexual relationship in a complex way, involving both psychosocial and biological factors, so closely interacting that it is difficult to assert the relative weight of hormonal and overall physical changes on psychosexual variations in BC survivors.

A multidisciplinary approach is needed, to offer the best consultation(s) for the individual case. Attention to the anatomy and function of the pelvic floor and pathophysiology of sexual response should become a mandatory part of a thorough clinical gynaecological and sexological examination, to give BC survivors the right to a full diagnosis and competent help.

New promising drugs and psychosexual interventions to improve women's and couple's sexuality after BC are under investigation. Meanwhile many practical suggestions can be offered from the lifestyle, pharmacologic, physiotherapic/rehabilitative and psychosexual point of view. Avoidance of the minimalistic and depressing wording "Be happy that you are alive" should be mandatory.

Finally, the fact that overall adjustment and QOL of BC survivors are positive in average 70–80% of cases should not mask a more painful truth: that this is true for many areas of QOL, *except* for all the dimensions of women's sexuality particularly in young BC women. An understanding and competent physician could help the woman and the couple to cope better with the tremendous strain of breast cancer, also from the sexual point of view: without giving up the sexual intimacy, that is such a critical part of QOL, particularly in younger women and couples. But it is difficult to provide an effective intervention, if there is no mention of a problem. That is why asking "How is your sexual life, now?" should become routine part of the oncological consultation in BC women, at least to make an appropriate referral, while promoting a better quality of life.

Appendix 1

Breast Cancer In Young Women: The Sexual Price

Executive Summary

Breast cancer may affect female sexual identity, sexual function and couple relationship in a complex way, involving both psychosocial and biological factors, by the many changes and challenges the woman has to face when breast cancer diagnosis and treatment disrupt her life and that of her relatives.

Psychosocial and biological factors are so closely interacting that it is difficult to assert the relative weight of hormonal and overall physical changes on psychosexual variations in breast cancer survivors. Physicians should improve their skill in understanding and listening to sexual concerns and in addressing the basic biological issues that breast cancer raises for female sexual identity, sexual function and sexual relationship.

Sexual Identity

Femininity, maternity, eroticism and social role all contribute to the perception of female sexual identity and may be variably affected by breast cancer diagnosis and treatment.

Femininity may suffer a major insult, for a number of biological reasons:

(1) *Body image*. Short-term impact depends on the type of surgery performed and the need or not of adjuvant radio- or chemotherapy and hormonotherapy. However, more conservative treatments do not appear to significantly modify quality of life nor women's sexuality in the long term.
(2) *Arm lymphedema*. "Arm problems" are quoted by 26 to 72% of the patients, according to the different arm symptoms (pain, pins and needles, numbness, skin sensitivity, swelling).
(3) *Iatrogenic menopause*. Younger patient are more vulnerable because a longer lacking period of adequate oestrogen and testosterone levels may affect the quality of ageing of

the brain and of sensory organs that are sexual targets and sexual modulators of sexual desire and central arousal.

(4) *Age*. Its weight is not limited to the potential impact of the menopause, but to the different individual and social tasks and goals of women's reproductive years, tasks that have different priority in different decades: young women should therefore be considered at higher risk of negative quality of life (QOL) outcomes after breast cancer and be offered a specific help.

Maternity: it may become the core of a major identity crisis for the 25% who is diagnosed during the fertile age. Since most breast cancer recurrences appear within 2 to 3 years after initial diagnosis, patients should be advised to postpone pregnancy for at least 2 years. If a patient has axillary node involvement, the recommendation to defer pregnancy should be extended to 5 years, but this recommendation is based on opinion only. Women treated for breast cancer who wish to become pregnant should be counselled that pregnancy is possible and does not seem to be associated with a worse prognosis for their breast cancer. Anyway, prior to attempting pregnancy, a breast cancer survivor should be referred for a full oncologic evaluation.

Eroticism: breast cancer may affect sensuality, sexiness and receptiveness through:

(1) *The loss of pleasurable sensations in the breast*, after surgery (partial mastectomy, in particular in case of breast reconstruction), which may reduce sexual arousal.
(2) *Menopause*: iatrogenic (chemotherapeutic) and/or nonhormonally treatable natural menopause may dramatically devastate the woman's sense of eroticism.
(3) *Depression and anxiety*, reactive to breast cancer, that may affect self-perception and sexual function (in particular sexual desire) via nonhormonal pathways, are reported in average 17 to 25% of breast cancer patients.
(4) *Social role* may represent an area relatively safe from breast cancer, particularly in well-educated women and ones with a strong and positive social role, except in the acute phase or in the more severe and aggressive cases.

Women carriers of BCRA mutations, who might consider bilateral prophylactic mastectomy, may have a specific iatrogenic impact of surgery on their self-image and femininity.

Sexual Function

The most common sexual symptoms in BC survivors, which can be referred to the loss of sexual hormones, altered body image and secondary to surgery or to other sexual disorders, are *loss of libido*, *arousal disorders* (arousal difficulties may be central, non-genital peripheral and genital), *dyspareunia* (lifelong or acquired, secondary to arousal disorder, it can be worsened by the defensive spasm of pubococcygeus muscle), *anorgasmia* (common after chemotherapy, worsening with time) and *loss of satisfaction* (pain and an overall disappointing sexual experience might also be responsible for the significantly reduced satisfaction reported by breast cancer survivors).

Attention to the anatomy and function of the pelvic floor should become a mandatory part of a thorough clinical gynaecological and sexological examination, to give BC survivors the right to a full diagnosis and competent help.

Couple Relationship

The fact that overall adjustment and QOL of breast cancer survivors are positive in average 70–80% should not mask a more painful truth: that this is true for many areas of QOL, *except* for sexual function and satisfaction. An understanding and competent physician could help the woman and the couple to cope better with the tremendous strain of breast cancer, also from the sexual point of view: without giving up the sexual intimacy, that is such a critical part of QOL, particularly in younger women and couples.

Treatment

Recommendations to ease the difficult sexual life of young BC patients are based most on clinical experience of the author and include: lifestyle intervention (daily exercise, appropriate diet, no smoking, breast self-massage, vaginal self-stretching and self-massage, resume sexual intercourse soon after BC surgery, if desired), physiotherapy, pharmacological interventions (nonhormonal and hormonal drugs, when appropriate and oncologically feasible), nonpharmacological methods (Eros clitoral therapy device, vibrators, lubricants), individual intervention (mindfulness-based interventions, psychoeducational intervention) and partner medical and/or psychosexual support.

Future hormonal treatment options may include estetrol (E4), ospemiphene [77], DHEA and testosterone (intravaginal gel or systemic): controlled studies are ongoing.

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Web Reference

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Male Breast Cancer

63

Laura Ottini and Carlo Capalbo

63.1 Introduction

Breast cancer (BC) is often mistakenly thought of as a female-only disease. Aside from the expected cultural explanations, the simple fact is that male BC (MBC) is rare. To date, in Western countries, MBC accounts for less than 1% of all cancers in men and less than 1% of all BCs, but its incidence is increasing [1, 2].

The earliest reference to BC in the Edwin Smith Surgical Papyrus from Egypt (3000–2500 BC) appears to have referred to a man [3, 4]. The first clinical description of an MBC case is in the early fourteenth century and is attributed to John of Arderne (1307–1390) who recorded the several-year evolution of nipple ulceration in a priest, with the subsequent development of BC [5]. Many years later, in 1842, Domenico Antonio Rigoni-Stern showed to the Congress of the Italian Scientist that the risk of BC was greatly increased in nuns compared to other women, and, intriguingly, he concluded his presentation saying that "the four men found to have died of BC were all priests" [6].

Because of its rarity, MBC is often compared with FBC, and our current understanding regarding MBC biology, pathology, and treatment strategies has been largely extrapolated from the more common female counterpart. Current epidemiologic and pathologic data, such as age-frequency distribution, age-specific incidence rate patterns, and prognostic factor profiles, suggest that MBC is like postmenopausal FBC [7]. Although MBC resembles postmenopausal FBC, clinical and pathologic characteristics of MBC do not exactly overlap FBC. Compared with FBC, MBC occurs later in life with more advanced stage, lower histologic grade, and more frequent estrogen receptor (ER)- and progesterone receptor (PR)-positive (ER/PR+) status [8, 9]. Higher stage and advanced age at diagnosis are negative prognostic factors, and, although rare, MBC remains a substantial cause for morbidity and mortality in men.

There is very little research on the management and care of MBC, and, due to the low incidence of MBC, studies are often small and underpowered. Thus, to date, therapy has been based largely on biomarkers developed for FBC, and MBC treatment generally follows the same indications as postmenopausal FBC. The outcome for men is the same as women stage for stage, but overall the prognosis tends to be worse in MBC [7]. This is usually due to a delay in presentation, leading to a large proportion of patients presenting with advanced-stage disease, and older age at diagnosis, leading to the coexistence of possible comorbidities. Overall, BC mortality and survival rates have improved significantly over time for both male and female BC, but the improvement for male is smaller if compared to female patients [9]. The different treatment outcomes may suggest a non-appropriate utilization of treatment options and a possible existence of different underlying pathogenetic mechanisms between male and female BC.

Today, thanks to the increasing number of collaborative studies and to the combination of data derived from new high-throughput technologies, we are now able to hypothesize that despite the similarities between female and male BC, MBC could be rather considered a distinct pathology.

63.2 Epidemiology

MBC incidence varies greatly in different geographical areas and ethnic groups and is correlated with FBC incidence worldwide [10]. Indeed, the worldwide variation of MBC resembles that of FBC, with higher rates in North America and Europe and lower rates in Asia [11]. A substantial high proportion of MBC cases have been reported in Africa, particularly in Uganda and Zambia [12]. These relatively high rates have been attributed to endemic infectious diseases causing liver damage leading to hyperestrogenisms. Overall, black men have a higher incidence of MBC (1.8 per 100,000)

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compared to the average incidence. By contrast, the annual incidence of MBC in Japan is significantly lower (5 per 1000,000) than the average incidence (1 per 100,000), comparable to the lower than the average incidence of FBC in this country [8, 13].

MBC incidence is increasing [2]. Data from the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database indicates that in the USA the incidence of MBC has been increasing during the last 20 years, from about 1 per 100,000 to around 1.2 per 100,000 (SEER 13 Registries Database). Data from the United Kingdom Association of Cancer Registries (UKACR) have reported that in the UK, MBC incidence is on the increase and parallels US data over a similar period of time [14].

Age-specific incidence rates for MBC increase linearly and steadily with age with a peak incidence in the late 60s. In contrast, FBC increases rapidly until around age 50 and then increases at a slower rate for older women (Fig. 63.1). MBC cases are diagnosed at a more advanced age than FBC. Age of BC presentation in males is mostly in the late 60s, which is about 10 years greater than in female. In the SEER database, the median ages at diagnosis of BC were 68 and 61 years in males and females, respectively. The differences in incidence rates and time trends between males and females may reflect gender-related differences in risk factors and/or different underlying pathogenetic mechanisms. On the other hand, the correlation between male and female BC incidences indicates the existence of common risk factors for BC in both genders.

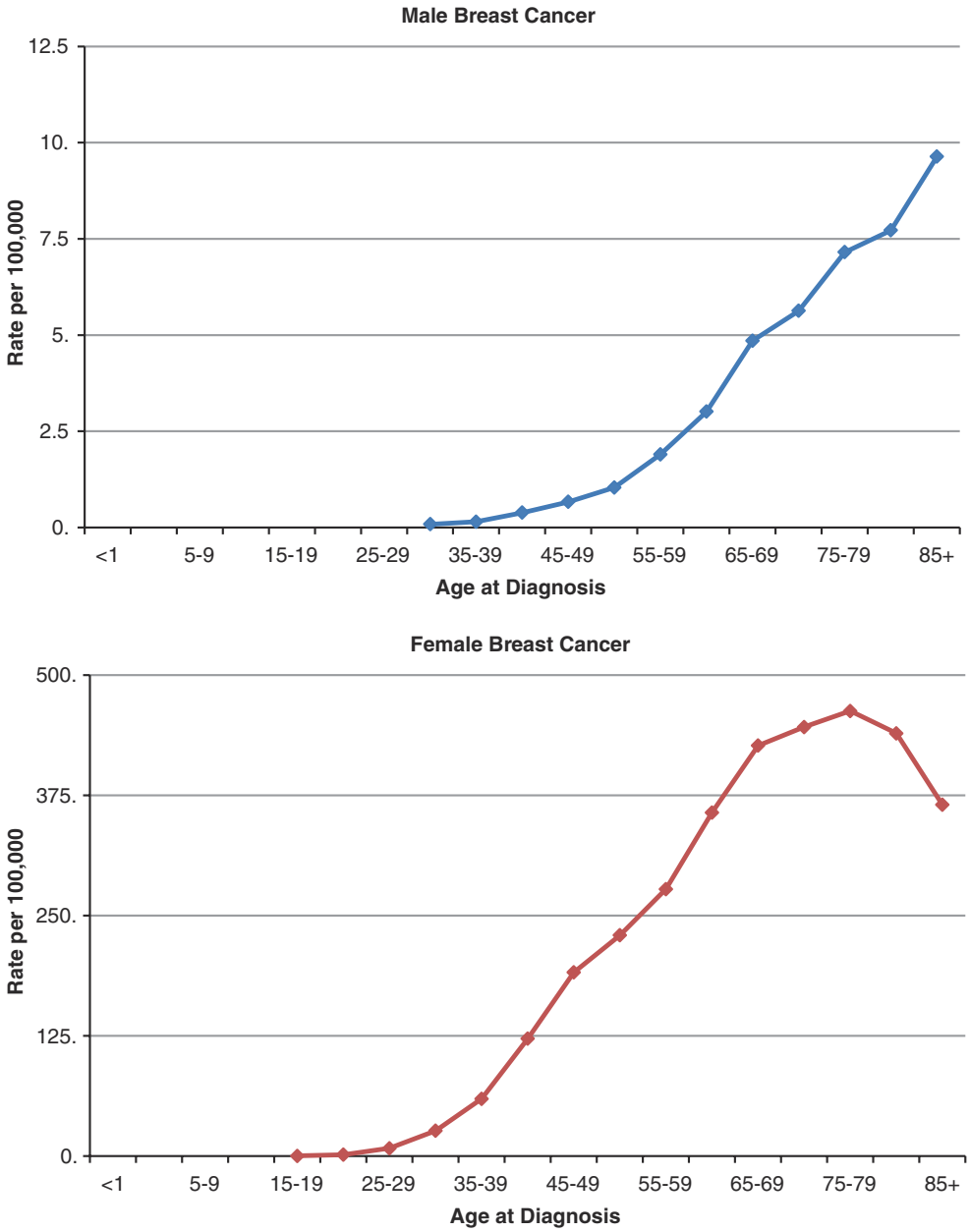

Fig. 63.1 Age-specific incidence rates (per 100,000) for male and female invasive breast cancer in white US population. Data source: SEER 18, years 2003–2012

Table 63.1 Risk factors for male breast cancer

Risk Factors	Genetic	Hormonal	Environmental
Well established	Breast cancer family history	Increased estrogen exposure	Radiation
	BRCA1/BRCA2	Deficiency of testosterone	
	Klinefelter's syndrome	Testicular disease	
Possible	*CHEK2*	Obesity	Heat
	PALB2	Diabetes	Electromagnetic fields
	SNPs	Liver damage	Polycyclic aromatic hydrocarbons
Suspected	Cowden syndrome	Gynecomastia	Alcohol
	Lynch syndrome		Tobacco

63.3 Risk Factors

Risk factors for MBC include genetic, hormonal, and environmental factors (Table 63.1).

63.3.1 Genetics

About 20% of male patients with BC have positive family history for BC. Men with a positive first-degree family history have a twofold increased risk of BC. The risk increases with increasing numbers of first-degree relatives affected, and a particularly enhanced risk (more than ninefold) is reported for cases with both an affected mother and an affected sister [15]. About 2% of male patients with BC develop a second primary BC, and more than 20% develop a second non-breast tumor, more frequently prostate, colon, and genitourinary cancer. Men who have family members with Cowden syndrome and Lynch syndrome are also at increased risk for BC [16, 17]. As both these syndromes and MBC are rare, it is difficult to determine as to whether germline mutations in these genes increase the risk of MBC.

Mutations in the two major high-penetrance BC genes, *BRCA1* and, predominantly, *BRCA2*, account for approximately 10% of MBCs, outside populations with *BRCA* founder mutations [18]. In Icelandic population, for example, the *BRCA*2 999del5 founder mutation is implicated in about 40% of MBC cases [19]. Mutations in *BRCA1* and *BRCA2* genes are often found in MBC patients who have multiple cases of breast and/or ovarian cancer in their family, but have also been found in MBC patients with no breast and/or ovarian cancer family history. This evidence and the rarity of the disease indicate that every man who has BC should be routinely screened for *BRCA1* and *BRCA2* mutations, regardless of family history, which could prove to contribute invaluable genetic information to family members. Men with *BRCA1/2* mutations have an increased risk of BC compared with general population and develop BC at younger age compared with non-mutation carriers. The lifetime risk of developing MBC has been estimated to be in the range of 1–5% for *BRCA1* and 5–10% for *BRCA2* mutation carriers, compared with a risk of 0.1% in the general population [20, 21]. The median age at BC diagnosis in male *BRCA1* and *BRCA2* mutation carriers is earlier (62 years) than that of the general population (68 years) (SEER 13 Registries Database).

Two genes, *CHEK2* and *Partner/Localizer of BRCA2* (*PALB2*), both functionally related to *BRCA1* and *BRCA2* in DNA repair pathways, also play a role in MBC susceptibility as moderate-penetrance genes. In certain populations, the *CHEK2* 1100delC mutation confers an increased risk of MBC, particularly in *BRCA1* and *BRCA2* mutation-negative BC families. However, this association is not so evident in MBC cases unselected for family history, and the contribution of the *CHEK2* 1100delC variant to MBC predisposition varies from one ethnic group and from one country to another [22–24]. Mutations in *PALB2* have been reported in families with female and male BC cases [25–27]. A fourfold higher frequency of MBC among relatives of *PALB2* mutation carriers has been shown. BC risk estimates for male *PALB2* mutation carriers still remain to be precisely assessed; however, *PALB2* may have an important role in MBC predisposition, at a comparable extent as for FBC [28].

A number of single nucleotide polymorphisms (SNPs) are implicated as low-penetrance alleles in MBC genetic predisposition [29]. The majority of SNPs that are associated with MBC risk are the same as those associated with FBC risk, but it appears that the magnitude of risk conferred by them differs between the two diseases. In particular, two SNPs (rs3803662 and rs1314913) have been identified significantly associated with MBC risk [30]. The rs3803662 localizes to the TOX High Mobility Group Box Family Member 3 (*TOX3*) gene (mapping to 16q12.1) and is a known modifier of FBC risk [31]. As shown for rare variants in *BRCA2* and *CHEK2* genes, the association with risk for this SNP is greater in males compared with females [30]. The rs1314913 SNP, located in intron 7 of the *RAD51B* gene (mapping to 14q23-24.2) belonging to RAD51 DNA repair family, is specifically associated with increased MBC but not FBC risk, supporting defined difference between male and female disease [32]. While the risk associated with these SNPs is low, they are likely to be

responsible for a substantial percentage of hereditary and sporadic MBCs because of their high frequency in the population.

63.3.2 Endocrine

MBC is recognized as being a hormone-dependent malignancy, and it is widely accepted as an estrogen-driven disease, specifically related to hyperestrogenism [33]. Clinical disorders associated with imbalanced estrogen/androgen ratio that result in abnormal estrogen exposure represent a major risk. Men affected with Klinefelter's syndrome, a rare congenital condition characterized by a 47 XXY karyotype and high levels of estrogens and low levels of testosterone, have been shown to have 20- to 50-fold increased MBC risk [34].

Other medical conditions linked to altered endogenous hormones including diabetes, orchitis, cryptorchidism, and gynecomastia have possible influences on MBC risk [35]. In addition, liver disease, leading to hyperestrogenism, emerged as a significant risk predictor of MBC, particularly among black men [36]. Conditions increasing exposure to estrogen or decreasing exposure to androgen, such as the long-term use of antiandrogens and estrogens in the treatment of prostate cancer, the exogenous administration of estrogen to transsexuals, or abuse of steroids for physical performances, have also been implicated as causative factors for MBC [37–39]. Elevated risks of MBC have also been related to a variety of other conditions associated with altered endogenous hormones, with the most consistent association being observed with obesity [40, 41]. Obesity is associated with an increased risk of postmenopausal FBC, presumably through peripheral conversion of androgens to estrogens. In men, obesity is associated with decreased testosterone and sex hormone-binding globulin levels but increased estrogen levels [42, 43], thus leading to greater estrogen bioavailability.

63.3.3 Environmental and Lifestyle Risk Factors

Ionizing radiations, occupational exposure to heat, and electromagnetic radiation have been considered as possible causal cofactors in the etiology of MBC [12]. Additional clues to a potential importance of other occupational exposures derive from studies showing elevated MBC risks among men exposed to polycyclic aromatic hydrocarbons [18, 44]. Some lifestyle factors have also been involved in the etiology, including alcohol and tobacco exposures, although results have varied across investigations [45].

63.4 Histopathology

63.4.1 Histology

The histology of BC occurring in men is generally similar to that occurring among women although the distribution of the histological types is different. The majority of MBCs are invasive ductal carcinoma (more than 80%) followed by ductal carcinoma in situ (about 10%). Lobular histotype is much less common in men (1.5%) than in women, due to the absence of terminal lobules in male breast tissue [46, 47]. Lobular histotype has been reported in association with Klinefelter's syndrome and, rarely, in men with no previous history of estrogen exposure. The rare tumor types, such as medullary, tubular, mucinous, and squamous carcinomas, are also reported in men although they are rarer than in women. On the other hand, papillary carcinoma is more frequent in men than in women. Both Paget's disease and inflammatory carcinoma are observed with equal frequency (about 1%) in both genders [12]. The majority of MBCs are low histologic grade [46, 47]. Data from the SEER 13 database show that 13% of MBCs are Grade 1 (G1), 50% Grade 2 (G2), and 39% Grade 3 (G3) tumors (Table 63.2) [43].

63.4.2 Biomarkers

The great majority of MBCs are ER+ and PR+ with more than 90% of tumors being positive for ER and about 85% for PR (SEER13, [43]). As reported for FBC, the percentage of men with ER+ BC significantly increases with patient age [1, 8]. Rates of androgen receptor (AR) expression in MBC have been variable in different cohorts, ranging from 39 to 95% [12, 48, 49]. Data about HER2 expression in MBC are inconsistent most likely because of small studies and of not standardized technical approaches. Studies performed by using both immunohistochemical (IHC) and fluorescence in situ

Table 63.2 Histopathology of male breast cancer

Histology	%
Invasive ductal carcinoma	85–95
Ductal carcinoma in situ	5–10
Invasive papillary	2–5
Lobular	1–1.5
Medullary	2
Mucinous	1
Paget's	1
Biomarkers	**%**
ER	90
PR	85
AR	39–95
HER2	15

hybridization (FISH) analyses report HER2 overexpression in about 15% of MBCs [50, 51].

Based on immunophenotypic characteristics, MBC can be classified into different molecular subtypes [50, 52]. Luminal A (ER+ and/or PR+, HER2-) subtype has been reported as the most common subtype in MBC (more than 85%), whereas Luminal B (ER+ and/or PR+, HER2+) subtype has been less frequently observed (about 12%). Triple-negative (ER-, PR-, HER2-) subtype and HER2-positive (ER-, PR-, HER2+) subtype have been rarely identified in MBC (about 1%).

63.4.3 BRCA1/2 Male Breast Cancer

Generally MBC presents with lower histologic grade tumors than FBC. In contrast, MBC associated with *BRCA2* mutations presents with higher histologic grade compared both with FBC in *BRCA2* mutation carriers and with MBC in the general population from SEER [53]. In particular, high histologic grade breast tumors are more frequent among male *BRCA2* mutation carriers diagnosed at younger ages (below 50 years) than among those diagnosed at older ages. Thus, the identification of a specific *BRCA2*-associated phenotype suggestive of an aggressive behavior may define a subset of MBC patients (i.e., patients with high-grade breast tumors and with young age at diagnosis) who might particularly benefit from adjuvant chemotherapy. A similar trend is also observed for *BRCA1* mutation carriers. Overall, *BRCA1/2* MBCs display distinct pathologic characteristics compared to *BRCA1/2* FBCs. These findings should lead to the development of gender-specific risk-prediction models and guide clinical strategies appropriate for MBC management.

63.5 Clinical Presentation and Diagnosis

Men with BC are more frequently diagnosed at a more advanced age and with a more severe clinical presentation, with greater tumor size and a more frequent lymph node involvement, than women with BC. In general, this is thought to reflect diagnostic delay in a population unaware of its risk and not appropriately encouraged to undergo routine BC screening. Despite increasing awareness of MBC, there remains an average delay from the onset of symptoms to diagnosis of 6–10 months [54].

The most common symptom of BC in men is a painless lump. The majority of MBC patients present a palpable subareolar mass. Because of the unique anatomy of the male breast, characterized by the presence of the rudimentary breast ducts located directly beneath the nipple, other initial symptoms may include nipple involvement such as retraction, ulceration, discharge, and bleeding [54]. BC primaries in men more often have locoregional metastasis at presentation. More than 40% of MBC patients present with stage III/IV disease, often due to early chest wall spread. Clinically suspected axillary nodes are identified in about 40% of patients at the time of diagnosis [46]. Bilateral involvement is rare, less than 2% of MBC cases [55].

Clinically suspicious lesions identified by palpation are evaluated with mammography and/or ultrasonography scans to select patients who will undergo to further examination generally made by fine-needle aspiration (FNA) or core biopsy. The primary differential diagnosis of a breast mass in a man includes gynecomastia that affects 30% of healthy men [56]. The evaluation of the extension of disease and stage classification follows the guidelines for FBC.

63.6 Prognosis and Survival

Prognosis depends upon tumor size, histologic grade, nodal status, and hormone receptor status. Stage classification of male patients with BC is similar to that of female patients, according to AJCC or UICC guidelines. As postmenopausal FBC, in general MBC have more favorable prognostic factor profile, including low histologic grade and hormonal receptor-positive expression [9]. Despite this, men experience worse prognosis than women, probably due to both an advanced stage and older age at diagnosis. As in FBC, the most important prognostic indicators are tumor size and lymph node status. Men with breast tumor diameter > 2 cm have a 40% higher risk of mortality than men with tumors <2 cm. Similarly, men with lymph node involvement have a 50% higher risk of mortality than men without lymph node involvement. Furthermore, an increasing number of lymph node metastases are positively correlated with a poorer prognosis [1].

Compared with women, overall survival rates are lower in men; however, when adjusted for older age at diagnosis and poor life expectancy, the relative survival rates are quite similar for men and women [9]. This likely reflects the influence of serious comorbidities as result of an older age at diagnosis. The overall 5- and 10-year survival rates of MBC patients are around 60% and 40%, respectively. When grouped by stage at presentation, overall survival rate is 78% and 55% for stage I, 66% and 39% for stage II, and 39% and 21% for stage III disease, at 5 and 10 years, respectively [57]. Overall, survival rates differ significantly according to race/ethnicity. Compared to white men, black men have features of more aggressive disease as higher histologic grade, larger tumor size, and higher rate of nodal involvement. Worse prognosis in black men after

adjustment to clinical, demographic, and treatment factors has been shown [58].

BC mortality and survival rates have improved significantly over time for both male and female BC, but the relative improvement was less significant for men compared with women [7]. Decline in FBC mortality rates are attributed to adjuvant systematic therapy and screening mammography. Decline in MBC mortality would likely reflect just the impact of adjuvant systematic treatment since men do not receive screening mammography. However, the improvement for male is smaller if compared to female BC patients, suggesting underutilization or non-appropriate utilization of adjuvant therapy.

63.7 Oncogenetic Counseling, Screening, and Surveillance

Genetic counseling should be offered to MBC patients based on their increased risk of *BRCA* mutations, particularly in the context of a family history of breast/ovarian cancer. The National Comprehensive Cancer Network (NCCN) recommendation indicates that all MBC patients should be offered genetic counseling and testing based on their risk of carrying a deleterious mutation that might be relevant to their own care or the care of their family members. Risk assessment models to estimate the risk of carrying a *BRCA* mutation, such as BRCAPRO, have been validated for use in male patients [59–61].

Because the age-standardized incidence of MBC is only 1/100,000 person-years with lifetime risk of about 1/1000, there is no role for breast screening in the general male population. On the other hand, screening for BC in men at higher BC risk, including those with *BRCA1/2* mutations, strong family history of BC, such as affected mother and/or sister, Klinefelter's syndrome, or transgenders [62, 63], should be undertaken and should be available preferably in a clinical trial. Extrapolating from FBC, NCCN recommends that men at higher BC risk have a clinical breast exam every 6 to 12 months and consider having a mammogram at age 40. However, as the value of breast imaging remains uncertain in men, mammography and ultrasound should be considered. Men with a *BRCA1/2* gene mutation should also have prostate cancer screening starting at age 40. Men at higher BC risk should also be aware of the warning signs of BC and should be taught for breast self-examination.

The risk of a new BC is higher in MBC survivors. MBC patients had a 30-fold increased risk of developing a contralateral BC, and this risk is greatest in men who were younger than 50 years at BC diagnosis. Thus, male survivors of early stage BC could benefit most from breast screening. MBC survivors are also at risk of certain non-breast second malignancies, prostate and colon cancer being the most common [47]. Thus, MBC survivors should be offered the same screening programs for non-BC as men in the general population, unless they are found to carry deleterious genetic mutations for which specific follow-up is recommended. Overall, there is a clear need for protocols for both screening and surveillance and, more in general, for information and support to men diagnosed with BC.

63.8 Treatment Options

To date, because there have been few evidences supporting a specific therapeutic approach in MBC, the majority of clinicians base their treatment recommendations on their personal experience with this disease and on the data of FBC. Indeed, the small numbers of MBC seen in any unit annually have precluded significant trials being carried out, and treatment of MBC often mirrors that of FBC despite some not negligible differences. Overall, with some minor variations, MBC treatment follows the same indications as female postmenopausal BC, with surgery, radiotherapy, and systemic therapy.

63.8.1 Treatment of Early-Stage Disease

63.8.1.1 Surgical Management

The goals of BC surgery include complete resection of the primary tumor with negative margins to reduce the risk of local recurrences and pathologic staging of the tumor and axillary lymph nodes to provide prognostic and/or predictive information.

In early-stage MBC patients, primary standard treatment is a modified radical mastectomy with axillary dissection [64]. This is primarily due to a paucity of breast tissue in men as well as the fact that in male breast, the tumor usually affects central quadrant. Locoregional disease control to this treatment is generally similar to those seen in women with BC. Conservative breast surgery has produced encouraging results for the treatment of early-stage MBC patients, although in males a larger tumor size and a higher rate of chest wall infiltration are found compared to female patients. Overall, breast conservation has also been used, and results have been similar to those seen in women with BC [65].

Surgical assessment of the axillary lymph nodes is an essential part of primary BC therapy; although men with

early-stage disease typically undergo axillary lymph node dissection, the use of sentinel lymph node biopsies is increasing [66, 67]. If the sentinel lymph node contains cancer, a full axillary lymph node dissection may be needed, depending on the size of the cancer in the lymph node as well as what other treatment is planned. In a recent SEER analysis, 19% of men were treated with lumpectomy [68]. Obviously, if breast-conserving surgery is done, it is usually followed by radiation therapy. External beam radiation is the usual type of radiation therapy for men with BC. This usually includes the chest wall where the breast was removed and, depending on the size and extent of the cancer, may include the underarm area, supraclavicular lymph nodes, and internal mammary lymph nodes.

Rates of contralateral preventive mastectomy in men who received a diagnosis of unilateral invasive BC nearly doubled between 2004 and 2011. In fact, in 2004, about 3% of men had contralateral preventive mastectomy while, in 2011, men who had contralateral preventive mastectomy were 5.6%, with a relative increase of 86.7% [69]. However, this increase has occurred despite the lack of evidence for a survival benefit from bilateral surgery.

Although breast reconstruction is an integral part of BC treatment plan and methods of reconstruction after mastectomy in women are well described, to date, postmastectomy deformity of the male chest has not received sufficient clinical interest. However, body image has emerged as an area of concern for MBC patients, thus deserving gender-specific attention [70].

63.8.1.2 Adjuvant Therapy

Currently, due to the rare occurrence of this disease, no controlled studies have compared adjuvant treatment options in MBC. In this setting, responses are generally similar but not identical to those seen in women with BC, and hormonal therapy has been recommended in all receptor-positive patients. In men with ER+, node-negative BC, endocrine therapy with tamoxifen is usually recommended [71]. In men with node-positive tumors, both chemotherapy and tamoxifen have been used and can increase survival to the same extent as in women with BC. However, there are limited data regarding adjuvant chemotherapy use; to date most clinicians offer either an anthracycline or anthracycline-taxane-based schedules to men who are classified at a high risk of recurrence based on the presence of axillary lymph nodal disease, larger tumors, younger age, and ER-negative tumors [72].

While the data supporting adjuvant chemotherapy in women are strong, there is little information on the effectiveness of adjuvant chemotherapy in men. However, most clinicians use similar guidelines for adjuvant chemotherapy in male and female patients. To date only one study has been performed to determine which male patients would derive benefit from adjuvant treatment as measured by the standardized 21-gene RT-PCR assay [73]. A similar gene expression profile has been found in male compared with female BCs. If these preliminary data will be confirmed by prospective studies, this testing may also be a reasonable help to guide the treatment of early stage, ER+, lymph node-negative MBC (Table 63.3) [74].

63.8.2 Treatment of Metastatic Disease

Advanced-stage disease at BC diagnosis is more frequently observed in men than in women; although a very small selected subset of patients with metastatic BC should be approached with curative intent, it is a generally held belief that once the patient with BC develops clinically detectable metastases beyond the regional lymph nodes, the disease is incurable [64]. The goals of care are to optimize both length and quality of life.

To date, clinical management of metastatic BC is similar in male and female patients. Treatment choice should take into account at least these factors: tumor burden, metastatic de novo or recurrence, hormonal receptors and HER2 status, previous therapies and/or toxicities, and disease-free interval. Given that the vast majority of men have ER+ tumors, hormonal therapy is the upfront approach. Tamoxifen has an established efficacy and toxicity profile in metastatic male BC, with an approximate 50% response rate, and is considered the standard first-line approach.

For male patients with hormone-refractory disease or rapidly progressing visceral metastases, chemotherapy can provide significant palliation. The type of chemotherapy is similar to the one used in the adjuvant setting, with anthracycline and taxanes as the backbone of the chemotherapy regimens. In particular, in the absence of medical contraindications, anthracycline- or taxane-based regimens, preferably as a single agent, would usually be considered as first-line chemotherapy for HER2-negative MBC, in those patients who have not received these regimens as adjuvant treatment and for whom chemotherapy is appropriate (Table 63.3). Similarly to female, in MBC patients pretreated with an anthracycline and a taxane and who do not need combination chemotherapy, single-agent capecitabine, vinorelbine, and eribulin are the preferred choices. The effectiveness of anti-HER2 agents in her2-neu overexpressing male breast cancer is unproven, but certainly it seems reasonable given the strong evidence in support of trastuzumab in women with breast cancer. Finally, no gender-specific trial have been designed so far testing mTor or CDK4/6 inhibitors.

Table 63.3 Drug options

Early stage			Advanced stage		
Chemotherapy regimens[a]:	Hormonal treatments[b]:	Target therapy[c]:	Chemotherapy regimens:	Hormonal treatments[d]:	Target therapy[e]:
AC (doxorubicin and cyclophosphamide)	Tamoxifen	Trastuzumab	Nab-paclitaxel	Tamoxifen	Anti-HER2 agents (trastuzumab, pertuzumab, TDM-1) in her2-neu overexpressing male breast cancer
AC or EC (epirubicin and cyclophosphamide) followed by T (doxorubicin and cyclophosphamide, followed by paclitaxel or docetaxel, or the reverse)	Aromatase inhibitors plus luteinizing hormone-releasing hormone agonist[f]		Doxorubicin	Luteinizing hormone-releasing hormone agonist with or without total androgen blockage (antiandrogen)	Bevacizumab
CAF (cyclophosphamide, doxorubicin, and 5-FU)			Paraplatin	Progesterone	plus LHRHa
CEF (cyclophosphamide, epirubicin, and 5-FU)			Cyclophosphamide	Aromatase inhibitors	CDK4/6 inhibitors
CMF (cyclophosphamide, methotrexate, and 5-FU)			Doxorubicin	Fulvestrant	
EC (epirubicin, cyclophosphamide)			Epirubicin		
TAC (docetaxel, doxorubicin, and cyclophosphamide)			Fluorouracil		
TC (docetaxel and cyclophosphamide)			Gemcitabine		
			Eribulin		
			Ixabepilone		
			Methotrexate		
			Vinorelbine		
			Paclitaxel		
			Docetaxel		
			Vincristine		
			Capecitabine		

[a]The role of taxanes and dose-dense regimen, however, remains to be elucidated
[b]The use of aromatase inhibitors for male BC is controversial; while these have been shown to increase disease-free survival in women, mixed results have been found in men
[c]There is currently few data on the benefits of trastuzumab in male breast cancer
[d]There is currently few data on the benefits of aromatase inhibitors and fulvestrant in male breast cancer
[e]The role of bevacizumab, mTor inhibitors and CDK4/6 inhibitors remains to be studied
[f]The effectiveness of anti-HER2 agents (trastuzumab, pertuzumab, TDM-1) in HER2 overexpressing male breast cancer is unproven but certainly seems reasonable given the strong evidence in support of trastuzumab in women with breast cancer

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Treatment of Central Nervous System Involvement

64

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64.1 Introduction and Epidemiology

Brain metastases (BMs) are the most common malignancy of the central nervous system (CNS), and their incidence has dramatically increased in recent years, mainly due to the improvements in systemic chemotherapy. This is particularly true for BM from breast cancer whose patients have, even with the best available treatments, a significant mortality and morbidity [1]. Approximately 10–15% of patients with breast primary tumors will develop BM during the course of disease [2], reaching 30% when considering autopsy series [3]. Brain relapse typically occurs within 2 to 3 years after the removal of the breast tumor [4]. The median survival after CNS metastasis in breast cancer patients is approximately 4 months, and the one-year survival rate is about 20% [5].

BMs are of particular interest because of the high mortality resulting from brain lesions and resistance to chemotherapies, mainly due to the inability of many conventional drugs to cross the blood–brain barrier (BBB). The lack of effective treatments for BM represents an important social health problem, and consensus on the therapeutic strategy, sequence of treatments, and combination therapies are still a matter of debate. Several studies have reported some factors associated with the development of BM such as young age, lymph nodal status, high tumor grade, and distant metastases. In addition, patients with metastatic triple-negative breast cancer (ER, PR, HER-2 unamplified) and HER-2-overexpressed subtype tend to develop brain metastasis at a higher rate [1, 6].

In recent years, besides the rise of new promising agents and combination therapies, a crucial role in the care of metastatic breast cancer is being played by the multidisciplinary approach in the development of efficacious treatment strategies, characterized by interaction and active cooperation among radiologists, surgeons, pathologists, radiation oncologists, and medical oncologists. Unfortunately, in routine clinical practice, metastatic breast cancer cases are discussed much less frequently than early breast cancer cases in multidisciplinary meetings. Therefore, the opportunity for a selected local treatment may be not always fully exploited [7].

The primary determinants of outcomes in breast cancer patients with BM are the tumor subtype and performance status of the patient [8, 9].

The most common symptoms experienced in patients with brain metastasis consisted of headache (35%), vomiting (26%), nausea (23%), hemiparesis,(22%), visual changes (13%), and seizures (12%). A majority of the patients had multiple metastases (54.2%). Cerebellum and frontal lobes were the most common sites of metastasis (33 and 16%, respectively).

A recent large retrospective study of 865 patients with BMs reported the median time interval from primary diagnosis to development of BMs, as well as median survival following the diagnosis of BMs, to be shortest in TNBC (27.5 months and 7.3 months, respectively) and HER-2-positive disease (35.8 months and 17.9 months, respectively) and relatively longer in patients with ER-positive/HER-2-negative (54.4 months and 10 months, respectively) and ER-positive/HER-2-positive disease (47.4 months and 22.9 months, respectively) [10]. Therefore, there is a great deal of interest in developing new therapeutic strategies for BMs, particularly in the TNBC and HER-2-positive breast cancer subtypes.

Clinician's arsenal against brain metastases from breast cancer involves supportive care, surgery, radiotherapy, radio-

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surgery, and chemotherapy, usually unified in a multidisciplinary cooperative effort. The choice of a treatment or a combination of treatments is dependent on number of lesions, location, size, symptoms, and patient performance status. In this chapter principal treatment options are presented together with a review of the literature.

64.2 Medical Treatment

There are currently no approved systemic chemotherapy regimens for the management of BMs. Traditional systemic chemotherapies included cisplatin, temozolomide, etoposide, capecitabine, epothilone B analogues, and various combinations of these agents. Except in the case of the platinum agents, the reported CNS objective response rates (ORRs) were typically modest, and the duration of benefit was short (<4 months) [11]. Trials of the platinum agents, in which the response rate was higher, are limited in relevance by differences in the patient populations compared with those in the modern era. In particular, patients in those trials tended to be less heavily pretreated in either the adjuvant or metastatic setting. More recently, Anders and colleagues reported results of a phase II trial of irinotecan plus iniparib in pretreated patients with TNBC [12]. Iniparib is a drug initially developed as a poly(ADP-ribose) polymerase (PARP) inhibitor but subsequently shown not to have any PARP inhibitor activity [13]. Nevertheless, clinical activity was observed, with a CNS clinical benefit rate of 30%, albeit with a median overall time to progression of just over 2 months [12]. Given that irinotecan is known to have CNS activity in other tumor types (e.g., glioblastoma), it is reasonable to postulate that most, if not all, of the activity observed in the trial was attributable to this agent. Further analyses are underway to identify factors predictive of response.

64.3 Novel Therapies

Trial design is a major consideration in the evaluation of novel therapies for management of BMs from breast cancer. There has been significant progress in the establishment of standardized guidelines to assess CNS response, progression, neurocognitive function, and quality of life [14, 15]. However, most novel agents evaluated for treatment of BMs are being assessed in the setting of disease that is refractory to systemic therapy and most often in patients who have received local therapies such as radiation. The majority of studies of BMs include small patient cohorts and lack a control arm, as there are no systemic therapies approved for use in this setting. Development of new systemic therapies for breast cancer—coupled with improvements in trial design, in imaging modalities, and in the definition and measurement of clinical endpoints—has led to a renewed interest in developing novel therapeutic approaches for BMs. Despite the increasing number of trials of systemic therapies specific for BMs, however, local therapy options remain the current standard of care for these patients.

64.4 Current Local Care Approach

In general, the initial management of patients with brain metastases depends on (a) the number, size, and location of brain lesions; (b) the presence or absence of neurological symptoms; (c) the patient's performance status and medical comorbidities; (d) the status of systemic metastases; (e) the availability of systemic treatment options; and (f) the patient preference. Initial management will include some combination of surgical resection, radiosurgery, and/or WBRT, depending on the above factors. Systemic therapy could be a consideration either on a clinical trial, in the context of minimal CNS disease burden with rapidly progressive extracranial disease, or in select, well-informed patients as an alternative to localized therapies with close follow-up (i.e., a patient with small, asymptomatic CNS lesions). Among patients who have developed subsequent CNS progression after initial standard therapy, options include surgical resection, WBRT, stereotactic radiosurgery (SRS), off-label use of systemic therapy, consideration of a clinical trial, or best supportive care. Options will vary based on prior treatments received, response to prior treatments, location and number of the new or progressive CNS lesions, and the other patient- and disease-related factors as listed above.

64.5 Surgery

Surgical resection has the potential to immediately resolve focal deficits or intracranial hypertension and to obtain a histological diagnosis. Surgery is particularly indicated in case of large solitary mass (>3–4 cm), if important perilesional edema is present, in case of relevant mass effect/midline shift, and in symptomatic patients [16].

One of the first studies on the role of resection was a randomized trial, in which surgery plus whole-brain radiotherapy (WBRT) was compared to WBRT alone demonstrating an improvement of overall survival from 15 to 40 weeks in patients with a single metastasis [17]. A successive trial randomized patients with a single brain metastasis to surgery plus WBRT or to WBRT alone. The combination (surgery + WBRT) obtained a longer overall survival (12 vs 7 months) and longer functional independency (9 vs 4 months) in patients with controlled extracranial disease [18]. Notwithstanding, patients with progressive extracranial disease had the same prognosis regardless of the treatment, as

subsequently confirmed by another group [19]. In case of multiple metastases, surgery can be considered, in particular if there is a principal lesion determining edema or neurological deficits [20]. Two dated studies have demonstrated that surgical resection of multiple metastases is not able to improve patient survival if compared to WBRT alone [21, 22]. In a more recent publication by Bindal et al., resection of all metastases has proven to increase patient survival if compared to incomplete resection (14 vs 6 months) [23].

Another relevant indication to surgery is the need to obtain a histological diagnosis, for example, in those patients who have no known primitive or if the time from primitive breast cancer diagnosis is excessive.

In regard to surgical technique, several neurosurgeons defend the use of "en bloc" instead of piecemeal resection. Rationale of en bloc removal is to prevent malignant cells seeding, thus reducing the risk of leptomeningeal dissemination and of tumor recurrence [22, 24, 25]. Another technical aspect involves the margin of the tumor. It has been demonstrated that metastases determine some amount of infiltration, usually not exceeding 5 mm from the solid tumor [26, 27]. Taking into account these considerations, actual gold standard for surgery should obtain en bloc removal of solid mass together with 5 mm margins when possible. In this effort, surgery can be guided by images, preoperative acquired (neuro-navigation) or intraoperative (ultrasound, magnetic resonance, fluorescence), that help in the identification of solid mass and its margin, also permitting to assess the degree of removal.

64.5.1 Stereotactic Radiosurgery

Stereotactic radiosurgery (SRS) is a form of radiotherapy based on delivering high dose of radiation focally to the tumor, thus spearing the healthy brain parenchyma. The principal advantage of this approach is to avoid the neurocognitive decline consequence of whole-brain radiotherapy while delivering a higher intensity of photon radiation to the volume target [28–30]. RTOG 90-05, in a prospective study, has identified the proper SRS doses depending on lesion size. The dose varies from 15 to 18 and 24 Gy for lesions of 31–40, 21–30, and 20 mm or less, respectively [31]. Numerous studies have reported that SRS alone or in combination with WBRT can achieve a high rate of local control [28, 32–34]. In a recent trial from EORTC (22952), SRS has demonstrated a local control rate at 1 year of 69%, while surgery of 41%, and SRS combined with WBRT of 81%. [35]. Kondziolka demonstrated that SRS can improve local control in multiple brain metastases (two to four) from 6 months with WBRT to 36 months with the addition of SRS. However, the overall survival was unchanged, depending mainly on extracranial disease [28]. The RTOG corroborated the effectiveness of combining SRS and WBRT in patients with one to three metastases and demonstrated an improvement of 1.6 months in overall survival in patients with one unresectable lesion [36].

Another active area of research is the use of SRS after surgical resection, in order to enhance local control. Soltys et al. observed 1-year control rate of 94% adding 2 mm margin to the surgical bed and 78% if no margin is added [37].

Whether SRS can substitute surgery in metastases treatment is still uncertain in terms of level 1 evidence. In routine practice most of institution decides the treatment plan on the basis of tumor size, location, clinical presentation, and patient comorbidity. Metastases greater than 3–4 cm and localized superficially are almost invariably treated with surgery, while those of 1–2 cm deep seated are treated with SRS. In case of lesions susceptible of both treatments, patient's symptoms are determining. Asymptomatic lesions can be treated with SRS, otherwise surgery is preferred. In case of patient with augmented surgical risk because of specific comorbidities, SRS could be favored [38].

64.5.2 Whole-Brain Radiotherapy

Over the years the mainstay of treatment has been WBRT, especially in case of multiple metastases. The first description of WBRT is from Chao in 1954 [39]; henceforward radiotherapy has gained a pivotal role in the palliation of brain metastases. WBRT has two main aims: destruction of the microscopic seeding of the brain and the control of macroscopic metastases. Over the years, a number of publications have studied WBRT application, fractionation schemes, and its influence on patient outcome [38]. Actually the treatment consists in opposed lateral fields that shield the nasopharynx, oropharynx, throat, and anterior orbits with doses and fractionation schemes that vary from 20 Gy to 40 Gy, delivered over 1 to 4 weeks. The scheme with the highest rate of local control (up to 70%) is from the control arm of RTOG 9508 [36] and provides 37,5 Gy in 15 fractions.

The combination of WBRT and surgery has proven to improve local control, also reducing distance failures within the brain. Patchell et al., in his randomized study, demonstrated that the addition of WBRT, after complete resection, could decrease intracranial failure from 70% to 18% and local recurrence from 46% to 10%; however survival was not increased [40]. In view of this, WBRT is advisable as adjuvant therapy after surgery in order to reduce intracranial recurrences.

On the other hand, side effects of WBRT have to be considered. Typically they include hair loss, headache, erythema, serous otitis media, fatigue, and neurocognitive dysfunction. Whereas most of them have little relevance, neurocognitive symptoms could be gravely debilitating. In

order to mitigate this effect, two solutions have been studied. One consists in administrating memantine, an N-methyl-D-aspartate (NMDA) receptor agonist that has demonstrated to significantly prolong the time needed for cognitive decline [41]. The other solution implicates an intensity-modulated radiotherapy in order to avoid the hippocampus, thus preventing the neural stem cell injury [42].

64.6 Supportive Care

Principal endpoints are to reduce perilesional edema, decreasing intracranial pressure, thus stabilizing acute neurological deficits and control seizures.

Dexamethasone is generally preferred because of the marginal mineral-corticoid effect and prolonged half-life. Starting dose is 4–8 mg/day [43], and in the majority of cases, it leads to relevant neurological improvement in 24–72 h. On the other hand, side effects from protracted steroid administration are common and are causes of further disability. Cairncross and Posner have reported that dexamethasone if used as single therapy produces approximately 1 month of remission of symptoms and an increase of the median survival if compared to patient not treated at all [44].

Anticonvulsant therapy is obviously indicated in patients who have experienced seizures, but there is still no evidence supporting prophylaxis in patients harboring brain metastases. Selection of antiepileptic drug must take into account potential drug interactions with steroids and chemotherapeutic agents. Phenytoin, carbamazepine, oxcarbazepine, and phenobarbital induce the cytochrome P450 reducing the half-life of corticosteroids and some chemotherapeutic agents, thus decreasing their efficacy. Furthermore steroids and chemotherapy could lead to subtherapeutic levels of anticonvulsants because of enzymatic induction and inhibition.

64.7 Summary

The treatment of central nervous system involvement by metastatic breast cancer is often a cooperative effort between neurosurgeon, radiation oncologist, and oncologist.

Relying on the actual literature, this synergistic management should contemplate the following points:

In case of a single brain metastasis, surgery can increase survival, especially if extracranial disease is controlled and the patient is symptomatic. Adjuvant SRS or WBRT can decrease local recurrence. If the solitary metastasis is near an eloquent area, SRS or WBRT alone can be suggested.

In case of multiple metastases (1–4), SRS with or without WBRT could enhance local control. WBRT should be delayed as much as possible to retard neurocognitive symptoms. Surgery can be used for the larger lesions.

In all other cases, WBRT remains the only solution in order to palliate symptoms and to improve local control.

The challenge ahead is to move some of the promising therapies from early-phase trials into a randomized phase II or III setting, to advance the standard of care for patients with BCBMs. To decrease the heterogeneity of responses, specific consideration will need to be given to the specific breast cancer subtype and to the identification of novel predictive biomarkers of response.

New markers for predicting BM occurrence in the primary tumor setting are urgently needed for the early detection of high-risk patients and to effectively prevent the formation of BM in those patients. To what extent "liquid biopsies" (i.e., analysis of CTCs or circulating nucleic acids) may contribute to this goal remains under investigation [45].

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Follow-Up of Patients with Breast Cancer

65

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Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death among females in the world. Thanks to the advances of screening, diagnosis and treatment, the number of BC survivors will continue to increase [1, 2]: according to GLOBOCAN worldwide estimates, 1.7 million new BC cases and 521,900 BC deaths were expected in 2012 [1], and this translated in a 5-year BC prevalence of 6.232.100 [3]. It is a huge population, with a significant number of older survivors and comorbidities, requiring prolonged clinical monitoring over time after primary treatment [4]. Therefore, for healthcare organizations, to provide facilities able to respond to this increased demand, focusing on multiple objectives, avoiding fragmentation and lack of coordination in care, is a major challenge.

65.1 Patterns of Relapse of BC

Risk of recurrence is function of time and clinical-biological factors. Familiarity about BC natural history and knowledge of diagnostic accuracy of available investigative tools are essential elements for proper management of BC follow-up. In a seminal paper, Saphner et al. [5] presented a retrospective joint analysis of seven adjuvant clinical trials conducted by the Eastern Cooperative Oncology Group from 1978 to 1988 including 3585 patients with a median follow-up of 8.1 years. In the whole population the hazard of recurrence peaked between years 1 and 2 after surgery, declined steadily until the interval between years 5 and 6, and afterward a slow reduction of the hazard occurred, but never reached zero even by year 12. Analysis according the estrogen receptor (ER) status showed that even if the hazard rate was higher for the ER-negative patients, the hazards for ER-negative and ER-positive patients crossed and were higher for the ER-positive group beyond 5 years. Indeed data from a study of International Breast Cancer Study Group with a very long follow-up confirm that patients with ER-positive BC maintain a significant recurrence rate even after more than 10 years of follow-up [6]. In a study comparing different temporal cohorts of patients, a similar pattern of disease relapse over time according to ER status persisted, even if the hazard rate of relapse improved significantly for both populations, possibly due to evolving adjuvant treatment [7]. Breast cancer is likely to metastasize to the local soft tissue, lymph nodes, bone, lungs, liver, and brain, but metastases can be observed in a variety of other organs, even unusual, and often in multiple sites at presentation. It has also been recognized that BC subtypes are associated with distinct patterns of metastatic spread and significant differences in survival after relapse. While the bone is the most common metastatic site in almost all subtypes, luminal/HER2 and HER2-enriched tumors are associated with a significantly higher rate of brain, liver, and lung metastases. Basal-like tumors have a higher rate of brain, lung, and distant nodal metastases, but a significantly lower rate of liver and bone metastases [8]. In case of late relapse, the bone and lung seem the most frequent sites of metastases, often detected after an unscheduled visit due to symptom emergence [9]. It's probably important to remember that infiltrating lobular carcinoma may exhibit a tendency to spread to unusual sites for BC, such as the gastrointestinal tract, genitourinary tract, peritoneum, retroperitoneum, and leptomeninges, making sometimes quite difficult differential diagnosis from primary cancers [10].

65.2 Surveillance After Primary Treatment

Even if there are no randomized data supporting intensive surveillance or any particular follow-up protocol, regular evaluation of patients with early BC after surgery and adjuvant treatment is recommended [11–14], according to patient needs and costs. The aims of follow-up are manifold [13, 15]:

- Early detection of cancer recurrence and early diagnosis of contralateral BC

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- Recognition, prevention, and management of disease or treatment-related complication, with quality-of-life (QOL) improvement
- Monitoring and supporting hormonal treatment adherence
- Promotion of general healthy lifestyles and attention to survivorship issues, with psychological support and information for relief of anxiety and fear of disease recurrence
- Update of cancer family history

65.3 Screening for Locoregional Disease Recurrence

Definition of locoregional recurrence (LRR) is not always consistent and clear in BC studies, sometimes making it difficult to compare study outcomes. Just recently the Maastricht Delphi Consensus addressed this issue providing with a clear definition of all types of recurrence in BC [16].

About 5% of patients develops LRR within 5 years with a local failure rate of approximately 1–2.5%/year [17]. A systematic review on 5045 patients showed that about 58% of isolated LRR after primary treatment for early BC were diagnosed during routine visits or routine tests, while 41% were discovered outside scheduled controls [18]. Patients with isolated LRR have substantial worse prognosis [19], and efforts for an early detection should be maximized, as it influences survival, with an estimated absolute reduction in mortality of 17–28% [20]. Risk factors for LRR after breast-conserving therapy or mastectomy are young age, pathological tumor status or nodal status, boost radiotherapy, and surgical margins. These factors, combined in a simple prognostic index, could help to identify patients with low (<7.5%), intermediate (7.5–12.5%), or high (>12.5%) risk of LRR at 10 years [21]. Molecular subtyping of BC can give further information to identify patients at increased risk of LRR [22], even if target therapy with trastuzumab may reduce also the risk of LRR in HER-2-positive disease [23].

65.4 Screening of Recurrence After Breast Conservative Surgery

Current guidelines suggest annual surveillance mammography after breast conservative treatment, even if a more frequent evaluation, albeit in the first years of follow-up, is also reported. Mammography detection rate of ipsilateral breast tumor recurrence is about 50%, equally detected by the patient or on physical examination [17]. Surveillance mammography sensitivity ranges 64–67% and specificity 85–97% [24]. Recurrent tumors detected by surveillance mammography are often smaller and have better prognosis [20]. Unfortunately, depending on previous surgery and radiotherapy, physical examination and imaging interpretation may be more complex and misled by false-positive results. Even if magnetic resonance imaging (MRI) has the highest sensitivity (86–100%) and specificity (93%) for routine ipsilateral BC detection [24], current guidelines recommend it mainly for patient at high risk of contralateral BC or in case of originally mammographically occult primary BC [17]. When mammographic, and/or sonographic findings are inconclusive in differentiating scar tissue from tumor recurrence, MRI is very useful as absence of enhancement was associated to an 88% negative predictive value for cancer [25]. The role of postoperative breast ultrasounds (US) in BC patients remains unclear and it's advised regularly only by the ESMO guidelines [13]. Annual supplemental US screening in intermediate and high-risk women with mammographically dense breasts resulted in an additional 3.7 cancers detected per 1000 screened women [26].

Accelerated partial-breast irradiation such as intraoperative radiotherapy (IORT) is now an option, both as anticipated boost radiotherapy and as the sole treatment modality in selected patients. Radiologist should be aware of imaging alterations possibly related to these treatment modalities such as seroma, architectural distortion, and cytosteatonecrosis with calcifications [27, 28]. Fat necroses and scar calcifications appear to be more frequent and larger after IORT than following external radiotherapy [28]. Multiple, relatively scattered, round calcifications at the lumpectomy site were described early after IORT delivery in relation to small tungsten particles from shielding devices and granulomatous reaction [27].

Similarly fat necrosis and oil cyst occur in about 75% of patients undergoing autologous fat grafting for breast reconstruction and augmentation. At mammography they generally manifest as round lucencies, and the thin walls of these oil cysts can calcify over time; US appearance may vary, but generally shows avascularity and circumscribed margins; breast biopsy and MRI may confirm diagnosis [29].

65.5 Screening of Postmastectomy LRR

Local-regional recurrence after mastectomy is generally discovered during routine clinical examination or by the patient. In case of mastectomy without reconstruction surveillance, physical examination is easier and targeted US scan of any palpable area may help [17]. Imaging surveillance of the reconstructed breast is generally not recommended. Mammography is very limited in women that have undergone a mastectomy with implant reconstruction, because there is minimal tissue between the pectoralis major muscle and skin to image. Moreover local recurrences are generally superficial to the reconstruction and easily detectable on physical examination [30].

Patients with breast implant can benefit of US for it has high sensitivity and specificity particularly in case of extracapsular rupture [31]. However, MRI represents the gold standard to evaluate capsule integrity, silicone extrusion in soft tissue, and local disease recurrence. The Food and Drug Administration (FDA) recommends MRI imaging screening for "silent" implant rupture 3 years after implantation and every 2 years thereafter [32]. Finally an additional reason for monitoring women with breast implants is related to the infrequent development of a primary breast anaplastic large cell lymphoma (ALCL) in the scar capsule adjacent to the implant [33]. Advances in reconstruction techniques have led to an increased use of autologous breast reconstruction; as autologous tissue is placed over the pectoralis major, concern may arise that physical examination is less sensitive to reveal chest wall recurrence. In the absence of clinical randomized trials or indication from available guidelines, uncertainty for follow-up of these patients exists. Locoregional recurrences after autologous reconstruction have been reported in 3.8%, located in the skin flap in 50% and in the chest wall in 50% [34]. They generally present as a palpable mass, irregularity, skin changes, or pain, but data on surveillance imaging are extremely limited [30, 34]. In a recent retrospective study on 541 patients who had mastectomy and autologous reconstruction and a median follow-up of 7 years, LRR was reported in 27 patients (5%), after a median time of 2.6 years. Clinical exam was regularly performed and detected LRR in 24 of 27 patients (88.9%). Additional mammography screening of the reconstructed breast added minimal benefit, detecting two recurrences on 25 biopsies that also were palpable [35].

A last point is that surgical management of the axilla has undergone to major changes in recent years. Preoperative accurate evaluation of the axilla with US and fine-needle aspiration cytology is cost-effective and mandatory [36] with a negative predictive value for axillary metastases of 79% and overall accuracy of 84% [37]. On the other side it's more difficult to recommend US axillary follow-up for patients who have had a negative sentinel node biopsy: axillary recurrence rate from pooled analysis is only 0.3% (median time interval 20 months, range 4–63 months) [38], and it's unlikely that monitoring of such patients with repeated US examinations is cost-effective in the absence of palpable nodes.

65.6 Screening of Contralateral BC

Different histologies from the primary tumor and the coexistence of an in situ component may corroborate the diagnosis of contralateral breast cancer (CLBC), while new techniques may help to distinguish it from metastatic disease with important prognostic implication [39]. Women with a personal history of breast cancer have a 1.5-fold to twofold increased risk for developing a CLBC compared with the general population [40]. In a large study with long-term follow-up, the annual risk of CLBC remained constant at approximately 0.75% per year after treatment, and cumulative risk at 20 years was 15.4%, without difference between primary ductal in situ or infiltrating carcinoma. The median time to any CLBC was 8.2 years (range 0.5–26.5 years) with the majority being infiltrating disease (83%) [41].

The incidence of CLBC decreased by approximately 30% since the early 1980s [42], mainly for ER-positive CLBC due to increasing use of adjuvant endocrine therapy [43]. The effect on prognosis resulting from the occurrence of a metachronous CLBC is probably limited, even if young patients and those diagnosed within 2–5 years from primary breast cancer could be at higher risk [40, 42]. Risk factors for occurrence of CLBC are, among others, strong family history of BC, BRCA mutations, young age, ER-negative disease, lobular histology, race/and or ethnicity, previous chest radiotherapy especially in young age, ataxia telangiectasia gene mutation, lifestyle, and reproductive factors [44, 45]. In a recent systematic review, a cumulative 5-year risk of CLBC for BRCA1 and BRCA2 mutation carriers was 15% and 9%, respectively, and the 10-year risk increased up to 27% and 19%, respectively, a remarkably greater risk than non-BRCA carriers (3% and 5%, respectively, at 5 and 10 years) [46]. Despite the current trend of increasing prevalence of contralateral prophylactic mastectomy, outside the subset of patients bearing BRCA mutation, risk-reducing surgery seems to have limited effect on survival [47].

Current guidelines suggest monthly self-examination and yearly mammographic evaluation for screening of CLBC, while breast MRI is generally regarded as an option for patients at high risk of bilateral BC, particularly BRCA mutated [11–14]. Data from nonrandomized study suggest that mammography detects approximately 45–90% of CLBC, with evidence of a potential survival benefit for asymptomatic/early-detected diseases [48–50].

Surveillance mammography has shown to reduce mortality in older patients (>65 years), after diagnosis of early-stage disease [51]. Unfortunately younger women tend to have CLBC more frequently detected by physical examinations or by self-diagnosis than older patients [52]. A recent health technology assessment concludes that surveillance with mammography every 12–24 months is likely to improve survival, with the need for stratification of patients to ensure maximum benefit and optimal use of resources being considered [53]. Better imaging techniques and tailored strategy are probably required beyond current recommendations to detect new CLBC cancer, mostly in younger patients and according to risk factors. We have limited information on MRI surveillance of women previously treated for BC, all from retrospective studies [54, 55], and inappropriate use

may result in low yield [55]. Detection rate ranges 9–12%. Sensitivity is high (91–100%) with a moderate specificity (about 80%) and positive predictive value (PPV) of 28–55%. In patient with personal and familiar history of BC, detection rate was 15% with a positive predictive value (PPV) of 50%; cancer detected with MRI was more likely to be DCIS or minimal cancer [56]. Outside BRCA mutation carriers, selective posttreatment MRI surveillance may have a place in patients at higher risk of recurrence, strong familiar history, or with anticipation of difficult detection such as young women, dense breasts, and mammographically occult primary cancer [54].

65.7 Screening for Distant Disease

Even if prolonged and long-term survival is described after treatment for MBC, particularly in case of oligometastatic disease [57], MBC is generally an incurable disease, and hence the main treatment goals are palliating, maintaining/improving quality of life, and possibly prolonging survival.

Two large prospective Italian trials investigating the impact of more intensive vs. a symptom-based follow-up strategy were published in 1994 [58–60], and both failed to detect an improvement in overall survival and QoL despite a greater number of metastases detected and a small diagnostic anticipation. In an additional Finnish study with a smaller sample size [61], 472 patients were randomly assigned to four arm exploring both frequency of follow-up visits (every 3 vs. every 6 months) and different follow-up tests (routine diagnostic tests including blood count, calcium, sedimentation rate, liver enzymes, and CA 15-3 at every visit, chest X-ray every 6 months, liver ultrasound, and bone scan every 2 years vs. no routine testing). Neither the frequency of visits nor the intensity of diagnostic examinations had any effect on disease-free or overall survival of patients and doubled the costs per detected recurrence from 4166€ to 9149€. A 2005 Cochrane Collaboration-sponsored meta-analysis confirmed these results both in the whole population and in subgroup analysis according to patient age, tumor size, and nodal status [62].

These data, however, refer to an era of less sophisticated diagnostic procedures and less efficacious treatment for advanced disease, and new trials urgently need to reassess this question [13].

The aforementioned guidelines and the 2007 update of recommendations for the use of tumor markers in breast cancer [63] claimed against their use during BC follow-up. Even if increasing level of CEA, CA 15-3, and, to a minor extent, alkaline phosphatase may announce breast cancer recurrence before a clinical or radiological evidence of disease, their definitive significance remains unknown [63, 64]. Fluorodeoxyglucose positron emission tomography-computed tomography (PET-CT) has high sensitivity (81%–97%) and specificity (52%–100%) in the assessment of women with suspected BC recurrence [65]. In a recent study PET-CT was coupled to CEA, CA 15-3, and CA 125 in case of elevated tumor markers as an intensified aftercare algorithm. Metastases were detected in 65.9% of patients, 13.6% had secondary malignancies besides BC, and 20.5% had no detectable malignancy. Limited disease was found in 24.1% of patients [66]. Thus, while FDG-PET scanning appears to be useful in case of suspected BC recurrence, no data support its role in routine breast cancer surveillance in asymptomatic patients.

65.8 Follow-Up in Special Populations

Older age does not seem a good reason for skipping surveillance mammography after primary treatment of early BC. Indeed in a US study, any additional surveillance mammogram was associated with a 0.69-fold decrease in the odds of breast cancer mortality. The protective association was strongest among women with stage I disease, those who received mastectomy, and those in the oldest age group [51]. Identifying frailty or pre-frailty may have prognostic significance, and providers should be aware of this [67].

Breast cancer under 40 years accounts for approximately 7% of all BC diagnoses. Routine mammography follow-up does not guarantee early detection of local recurrence or CLBC in young women with BC [68]. These young women have a number of particular issues specifically related to fertility and future pregnancies, menopausal, and psychosocial problems [69].

As far as male BC is concerned, there is limited information about the benefit of the contralateral breast screening mammography, MRI, or US [70], due to the relative rarity of the disease. A subset of men at higher risk of CLBC that could possibly benefit from screening mammography are younger men (<50 years at diagnosis), with strong family history of BC, BRCA carriers, Jewish ancestry, Klinefelter's or testicular disease, local exposure to radiation, liver disease, and estrogen treatment [70, 71]. Clinical and self-examination should be adequate in most patients as male BC generally presents as a palpable nodule [17, 47].

65.9 Second Non-breast Cancer

An increased risk of new non-breast malignancies has been reported among breast cancer survivors. Analysis of SEER data [72] indicates a 17.6% cumulative incidence of any second primary after BC at 25 years (including a 6.9% incidence of CLBC) and higher risk for black and younger women. Salivary gland, esophagus, stomach, colon, uterine

corpus, ovary, thyroid, and soft tissue sarcomas; cutaneous melanoma and acute nonlymphocytic leukemia (ANLL) were cancer types whose hazard was significantly increased. An individual predisposition or possible carcinogenic effects of adjuvant treatments may be factors involved in this often late-onset event.

A recent meta-analysis confirms that adjuvant radiotherapy is associated to a small but significantly increased risk of second non-breast cancer. After 5 or more years, relative risk (RR) was 1.12 overall, 2.53 for sarcomas, 1.53 for esophageal cancer, and 1.39 for lung cancer, while no association was seen for thyroid carcinoma [73]. However, the benefit of adjuvant RT on local control and OS far outweighs the risk of getting a radiation-induced second cancer 10–15 years after treatment [74].

Clinical studies seem not to find an increased risk of CLBC after adjuvant RT in BRCA mutation carrier, even if concern remains, particularly in young patients [75].

Noteworthy is the postradiation breast angiosarcoma, a rare but very aggressive complication with a latency of several years after treatment and generally exhibiting c-myc amplification [76]. The tumor presents as a cutaneous red-blue-colored lesion, whose appearance on the irradiated breast may be confused with the benign skin changes of radiation [77].

Perhaps the most feared adverse effect after adjuvant treatment is the acute myeloid leukemia (AML) or myelodysplasia (MDS). Two types of AML have been described: the first one is generally observed after exposure to alkylating agents, a mean latency period of 5–7 years, and is frequently preceded by a MDS phase. Chromosome 13 deletions or complete or partial loss of chromosome 5 or 7 is reported. A second type of AML, related to exposure to anthracycline-topoisomerase inhibitors, generally shows no latency period or MDS phase and different chromosomal alterations. Risk of AML after adjuvant treatment ranges 0.2–1.7%, in different studies [78]. In a recent large study on 20,063 stages I–III BC, most of which had received four cycles of an anthracycline and cyclophosphamide as part of adjuvant chemotherapy regimen with a median follow-up of 5.1 years, a marrow neoplasm (MN) was diagnosed in 50 patients (0.25%). Compared to no adjuvant treatment, hazard ratio for MN was 2.6 for radiotherapy only, 6.8 after surgery plus chemotherapy, and 7.6 after surgery, chemotherapy, and radiation. Cumulative incidence of MN increased with time being 0.24% at 5 years and 0.48% at 10 years [79]. Patients exposed to tamoxifen (TAM) have an increased risk of endometrial proliferation, hyperplasia, polyp formation, invasive carcinoma, and uterine sarcoma. The increased risk of endometrial cancer is limited to postmenopausal women. No risk increase or possibly a decreased risk has been observed with aromatase inhibitors (AI), and switching to an AI reduces TAM risk [80]. In the Early Breast Cancer Trialists' Collaborative Group meta-analysis of AIs vs. TAM [81], the 10-year risk was 0.4% in the AIs vs. 1.2% in the TAM group (absolute difference 0.8%, 95% CI 0.6–1.0; $p < 0.0001$). Continuing TAM after 5 years further increases risk: in the ATLAS trial, the cumulative risk of endometrial cancer during years 5–14 was 3.1% (mortality 0.4%) for women allocated to continue TAM vs. 1.6% (mortality 0.2%) for controls [82].

Even if uterine endometrial cancers after TAM treatment are often considered to be tumors with a good prognosis, data from a Dutch study shows that TAM-associated tumors may have less favorable histological features and a poorer survival, with a worsening prognosis in long-term users [83].

Premenopausal women have no known increased risk of uterine cancer and require no additional monitoring beyond routine gynecologic care. Postmenopausal women should be closely monitored for symptoms of endometrial hyperplasia or cancer, but routine transvaginal ultrasonography, endometrial biopsy, or both are not indicated in asymptomatic women. Pretreatment evaluation may identify patients at higher risk: the risk of incidence of atypical hyperplasia was 11.7% in the group with initial benign polyps vs. 0.7% in the group without lesions ($P < 0.0001$) [84].

65.10 Organizational Health Issues

A Canadian randomized clinical study was reassuring in showing similar outcome for patients allocated to receive, approximately 1 year after diagnosis, routine specialist follow-up in the clinic or from their own primary care physician (GP). Recurrence, deaths, recurrence-related serious clinical events, and health-related quality of life were similar in the two groups. However, only 58% of the screened patients accepted to participate in the study, and median follow-up was of only 3.5 years from randomization (4.5 years from diagnosis) [85]. In a cross-sectional study conducted on 145 patients, 73% of women were extremely satisfied of the follow-up provided by their GP [86], and in general GP showed willingness to assume exclusive responsibility for routine follow-up of patients [87].

65.11 Guidelines and Recommendations

Table 65.1 summarizes recommendation from some of the more recently updated follow-up guidelines. In general, guidelines are consistent in recommending to avoid surveillance radiographs, blood tests, and radionuclide scans in the asymptomatic patient and to prefer a symptom-directed assessment.

Table 65.1 Guidelines and recommendations for surveillance after primary treatment of breast cancer

Items	ASCO (2012 update) [11]	NCCN version 1.2016 [12]	ESMO 2015 [13]	AIOM 2015 [14]
Interval history and physical exam	Every 3–6 months for the first 3 years, every 6–12 months for 2 years, and annually thereafter	1–4 times per year as clinically appropriate for 5 years and then every 12 months	Every 3–4 months for 2 years, every 6 months from years 3–5, and annually thereafter	Every 3–6 months for the first 3 years. Every 6–12 months for years 4 and 5; annually thereafter
Mammography	Every 12 months (first posttreatment mammogram no earlier than 6 months after RT)	Every 12 months (wait 6–12 months after RT) Routine imaging of reconstructed breast not indicated	Annual mammography plus US recommended	Annual mammography (first 1 year after diagnosis or at least 6 months after RT)
Breast MRI	Not recommended for routine surveillance	Option in patients at high risk of bilateral BC (e.g., carriers of BRCA1/BRC2 mutations)	May be indicated for young patients, especially with dense breast tissue and genetic or familial predispositions	Not recommended except for BRCA mutation carriers
Laboratory and imaging tests	Not recommended	Not indicated. For women on AIs and treatment-related amenorrhea, estradiol and gonadotropin monitoring	Not recommended. Ultrasound can be considered in lobular invasive carcinomas	Not recommended in the absence of clinical indications
Breast self-examination	Monthly breast self-examination recommended			Monthly self-examination "could be performed"
Pelvic examination	Regular gynecologic follow-up recommended for all women; patients on tamoxifen should be advised to report any vaginal bleeding to their physicians	Annual gynecologic assessment for women on tamoxifen if uterus is present (routine US or biopsy not recommended)	Annual gynecological examination, possibly with a gynecological ultrasound, recommended for patients on tamoxifen	Regular gynecologic evaluation, including pelvic ultrasound and PAP smear, advisable
Bone health assessment		Bone mineral density at the baseline and periodically thereafter in women on an AI or with ovarian failure secondary to treatment	Regular bone density evaluation is recommended for patients on AIs	Periodic bone mineral density examination should be considered for patients on AIs
Blood cholesterol and triglyceride monitoring			Indicated for patients on ET	Periodic monitoring in case of treatment with AIs
Follow-up duration				No clear indication for interruption of yearly mammography. Referral to PCP at the end of follow-up in the specialist clinic
Coordination of care	By a physician experienced in the surveillance of patients with cancer and in breast examination. Possibility of transfer of care to a PCP 1 year after diagnosis in patients with early-stage BC. Continuity of care should be ensured			

Table 65.1 (continued)

Items	ASCO (2012 update) [11]	NCCN version 1.2016 [12]	ESMO 2015 [13]	AIOM 2015 [14]
Patient education	Counseling about the symptoms of recurrence	Educate, monitor, and refer for lymphedema management. Assess and encourage adherence to ET. Active lifestyle, healthy diet, limited alcohol intake, maintaining an ideal body weight Breastfeeding not contraindicated in case of pregnancy	Regular exercise and nutritional counseling for obese patients recommended Hormone replacement discouraged	
Symptom management and concomitant drugs		Warning about concomitant use of TAM and certain SSRIs. Use of hormones for birth control or osteoporosis discouraged (bisphosphonates or denosumab preferred)	Patients should have unlimited access to specialized rehabilitation facilities and services, to decrease the physical, psychological, and social "sequela" of breast cancer treatment	
Genetic counseling	Women at high risk for familial breast cancer syndromes should be referred for genetic counseling	Periodic screening for changes in family history		

MRI magnetic resonance imaging, *US* ultrasonography, *RT* radiotherapy, *AI* aromatase inhibitor, *ET* endocrine therapy, *PCP* primary care physician, *SSRIs* serotonin reuptake inhibitors

Compliance of physician with current guidelines has not been precisely evaluated, although some reports indicate a variable grade of discrepancy [88–94]. As pointed out by the recent 2015 ESMO guidelines, most available data for follow-up recommendations come from an era of less sophisticated diagnostic procedures and less effective treatments for advanced disease [13]. Different authors have evaluated the issue of follow-up personalization according to locoregional risk of recurrence [95, 96] or contralateral cancer [97], and a feasibility study showed that implementation of a tailored follow-up program according to risk of recurrence may permit to decrease the number of visits for low-risk patients [98]. Moreover current guidelines do not take into account that BC is a heterogeneous disease and that different molecular subtypes behave differently, and this could challenge also the "one-size-fits-all" follow-up strategy. New trials are urgently needed to reassess this question [99, 100], but we are aware of only one ongoing study from Japan exploring if intensive postoperative surveillance could be worthy in high-risk EBC [101].

Meanwhile the scenario could be quickly modified by advance in imaging [102] and laboratory diagnostic monitoring for minimal residual disease [103, 104].

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Part X

Investigating New Drugs and Immunological Agents in Breast Cancer

Targeting Immune Checkpoint

66

Angela Esposito and Giuseppe Curigliano

66.1 Introduction

Tumor cell transformation prompts to activation of adaptive and innate immune responses, which had a crucial role in eliminating and controlling early cancer growth. Over the past 10 years, there has been a greater understanding of the immune response to tumors which has led to the development of a huge number of immunotherapeutic strategies [1, 2]. The immune system plays a dual role in cancer: it not only can suppress tumor growth by destroying cancer cells or inhibiting their outgrowth but also promotes tumor progression either by selecting for tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth. The conceptual framework called "cancer immunoediting" integrates the immune system's dual host-protective and tumor-promoting roles. Nonetheless, numerous studies have shown that tumors can be recognized and contained for extended periods of time by the immune response through the concerted action of the innate and adaptive immune responses [3]. Agents such as the immune checkpoint inhibitors have demonstrated to induce a response in a number of solid malignancies [4], but their therapeutic benefit has not been seen in all cancer types. The initial enthusiasm for immune checkpoint inhibitors is mainly based on results obtained in melanoma, lung cancer, bladder cancer, and renal cell carcinoma [5]. But also in breast cancer (BC), preliminary data from the first clinical studies is encouraging.

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66.2 Role of Immunotherapy in Breast Cancer Treatment

Evading immune destruction should be considered an emerging hallmark of cancer. The knowledge of the underlying principles of tumor biology and immunology, enhanced by recent insights into the mechanisms of immune recognition, regulation, and tumor escape, has provided new approaches for cancer immunotherapy [6]. Highly immunogenic cancer cells can be eliminated in immunocompetent hosts as a result of the "immunoediting" process. Weakly immunogenic variants can grow and generate solid tumors [7]. A variety of tumor-infiltrating cells, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), and activated (type 2) macrophages (M2), are involved in the modulation of immune responses in cancer patients [8]. For example, an increased number of Tregs was found in blood and in the tumor microenvironment of patients affected by different tumors: it was demonstrated that Tregs suppress T-cell response and natural killer (NK) cell proliferation and function, thus interfering both with acquired and innate immunity [9]. In BC, recent evidence has demonstrated that immune-related factors play an important role in defining patient prognosis and their response to treatment. These include the extent of lymphocyte infiltration in tumor tissue [10] and a class of gene expression signatures [11], both of which have the potential to more precisely define patients' clinical evolution and identify patient subgroups with different sensitivities to standard treatments.

BC has not been traditionally considered immunogenic, as it does not occur at a higher incidence in the immunosuppressed populations who have been treated with immunosuppressive therapies [12]. However, it seems that, despite a weak influence on primary tumor growth, the immune system is effective in preventing BC metastases [13, 14]. The heterogeneous expression of tumor antigens within the primary tumor or its metastases, the modification of antigenic

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profile during the tumor progression, and the low levels of the antigen, major histocompatibility complex proteins, and other costimulatory proteins necessary to generate a strong immune response can explain this low immunogenicity. Moreover, the tumor microenvironment releases immunosuppressive factors that make the antigen presentation difficult and that have a negative impact on the immune response [15]. The interaction of the immune system with tumor cells in breast cancer seems to be breast cancer subtype specific. Triple-negative BC (TNBC) and HER2-positive BC harbor higher genomic instability compared to luminal A and B subtypes, leading to increased DNA damage or mutational load [16, 17]. Recent data shows that a higher mutational load elicits production of higher tumor-specific antigen levels and can produce stronger immune responses [18, 19]. When the immune system fails to eliminate all cancer cells, the less immunogenic cells survive and tumors can evade the immune system [20]. T-cell-inhibiting immune checkpoints have an important role in this escape. The binding of programmed death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on T cells with programmed death ligand 1 (PD-L1) and CD80, respectively, on tumor cells can strongly decrease T-cell activity. In addition aberrant expression of major histocompatibility complex II (MHC II) has been correlated to weak tumor immunity [21]. This demonstrates the tumor MHC II antigen presentation pathway is a crucial component of tumor immunity. The TNBC and HER2-positive BC subtype produce stronger immune responses and are hypothesized to be more dependent on these resistance mechanisms [19].

66.3 Immune Checkpoint Blockade

In recent years, the better knowledge of BC biology has provided an opportunity to develop some types of immunotherapy to overcome the relative non-immunogenic property of BC and improve immune response. Some molecules such as PD-1 and its ligand PDL-1, CTLA-4, and immune cells such as regulatory T (Treg) cells are involved in the induction of tolerance to antigens, and their upregulation is associated with increased risk of developing BC [22]. Recently, agents which stimulate T-cell function, by blocking immune checkpoints, have an emerging clinical interest. Checkpoint receptors, including CTLA-4 and PD-1, are upregulated on activated T cells and transmit inhibitory signals, which suppress T-cell proliferation and function [23]. CTLA-4 is a member of the CD28:B7 immunoglobulin superfamily, and it is normally expressed at low levels on the surface of naive effector T cells and Tregs [24]. After stimulation of a naive T cells, CTLA-4 is upregulated and competes with CD28 for B7 and, finally, leads to suppression of T-cell activity. Anti-CTLA-4 mAb facilitates T-cell proliferation and activation and abrogates the suppressive function of Tregs [25]. In addition to CTLA-4, PD-1 is a key immune checkpoint protein expressed on chronically stimulated T cells, which leads to the suppression of T-cell activity through interaction with its ligands, PD-L1 and PD-L2 [26]. Antibodies targeting PD-L1 or PD-1 have been shown to promote cytotoxic T-lymphocyte expansion [27] and tumor regression in many mouse tumor models [28–30]. Only two studies with CTLA-4 blocking agents in breast cancer patients have been performed. An exploratory study in 18 patients with predominantly hormonal receptor-positive early-stage breast cancer demonstrated a lowly increased ratio of CD8+ to Treg cells in tumor sample of patients who underwent mastectomy after pretreatment with ipilimumab and cryotherapy, while pretreatment with cryotherapy or ipilimumab alone did not increase this ratio. Cryotherapy causes a release of tumor antigen. It was therefore supposed that ipilimumab might augment the response against these antigens [31]. In another phase I study, the combination of tremelimumab and exemestane was explored in 26 postmenopausal metastatic breast cancer patients. Treatment was well tolerated. The best overall response rate (ORR) was stable disease for 12 weeks or more in 11 of 26 patients (42%). Treatment was associated with increased levels of peripheral CD4+ and CD8+ T cells that expressed the protein inducible costimulator (ICOS) of T-cell activation, a potential biomarker of immune activation resulting from blockade of CTLA-4 [32]. Two agents targeting the PD-1 and PD-L1 have been studied in breast cancer. Pembrolizumab (also known as MK-3475 and lambrolizumab) is a humanized monoclonal IgG4-kappa antibody that blocks PD-1 signaling [33]. In a phase Ib study, pembrolizumab was evaluated in 32 patients with PD-L1-positive metastatic TNBC. The overall response rate for 27 evaluable patients was 18.5% and the 6-month PFS rate was 23.3% [34]. 17.2% of the patients had at least one serious adverse event, and one patient died due to disseminated intravascular coagulation. MPDL3280A, a human IgG1 anti-PD-L1 monoclonal antibody with an engineered Fc receptor, preventing it from causing ADCC, has been evaluated in a phase I study in 27 pretreated patients with PD-L1-positive metastatic TNBC [35]. Among 21 efficacy-evaluable patients with a PD-L1 IHC 2 or 3 score (13 IHC 2 and 8 IHC 3), the unconfirmed RECIST response rate was 24% (95% CI, 8% to 47%). Response duration ranged from 0.1+ to 41.6+weeks. A phase III study in 350 metastatic breast cancer patients researching combination treatment with MPDL3280A and nab-paclitaxel is currently recruiting patients (NCT02425891). Anti-PD-1/PD-L1 agents are more effective in tumor types with higher mutational load, such as NSCLC and melanoma [36]. TNBC express high genomic instability due to double-strand DNA-repair deficiencies [16]. This subtype also has highest levels of PD-L1 in all breast cancer types [37]. The presence of pro-

grammed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer [38]. According to present thinking, this might increase effectiveness of checkpoint inhibitors in this subtype.

66.4 The Combination of Immunotherapy with Radiotherapy

Greater understanding of radiation therapy's effect on tumor cells and components of the tumor microenvironment has in turn evidenced the central role of the immune system, as highlighted by Lee et al., who described that in a mouse model, radiotherapy (RT) needs the presence of CD8+ T cells for post-RT tumor control [39]. The interaction of host immune system and proper antitumor activity can lead to immune-mediated rejection of non-irradiated metastatic lesions after irradiation of the primary lesion in a process known as the abscopal effect. The abscopal effect of radiation therapy is an event by which a primary tumor is irradiated and a response is observed at distant metastatic sites externally of the field of the radiation [40]. Preclinical evidence supported the hypothesis that the abscopal effect is mediated by the immune system [41], but the effect of radiation appears to be relatively weak and is rarely seen in clinical practice. Recently, there have been an increasing number of case reports showing the appearance of abscopal effects when radiotherapy is concomitantly administered with immune checkpoint inhibitors [42, 43], underlining that the radiotherapy treatment can lead to an immune response which is augmented by the immune-modulating agents [44]. The critical role of radiation is to induce the release of tumor immunogenic antigens responsible of the augmented pool of intracellular peptides for cross presentation; in this way the radiation creates an "in situ vaccination" [45]. During combinatorial treatment between RT and immunotherapy, the tumor-specific immune response is elicited and intensified subsequently. Preclinical models showed that radiation can augment tumor-specific antigen–MHC complexes, upregulate antigen cross presentation in the draining lymph node, and increase T-cell infiltration into tumors [46]. Of particular interest is the combination of anti-PD-1 antibodies with radiotherapy. Supporting data of the efficacy of this combination has been demonstrated in a murine model of breast and colorectal carcinomas, where augmented tumor control was showed [43]. Actually, many early-phase clinical trials combining immune checkpoint inhibitors with radiotherapy are ongoing. A number of these studies are examining the combination of stereotactic radiotherapy with PD-1 or PD-L1 inhibitors in patients with oligometastatic disease [47, 48]. The optimal timing of administration, the duration, the sequence of the immune-modulating agents with RT, and the appropriate patient populations are still not elucidated.

66.5 Immune-Related Toxicity of Immune Checkpoint Inhibitors

Toxicity is a major issue for the new cancer immunotherapy. Ipilimumab and other immunomodulatory drugs have been associated with several immune-related adverse events (irAEs), most of them related to the infiltration of highly activated CD4 and CD8 T cells and the increased production of inflammatory cytokines in normal tissues [49]. The most common irAEs related to the use of anti-CTLA-4 antibody involve the gastrointestinal tract, skin, liver, and endocrine system [50]. These effects are reported in up to 60% of patients treated with ipilimumab, with severe toxicities (grades 3 or 4) in about 10%–15% of patients [51]. They can appear at various times after anti-CTLA-4 treatment. The average timelines for irAEs are 2–3 weeks for dermatologic events, 6–7 weeks for gastrointestinal and hepatic events, and 9 weeks for endocrine events [52]. The presentation of irAEs can vary from insidious to sudden and can be confused with other known autoimmune conditions. Usually, irAEs were reversible, but in rare cases, they may be severe and life-threatening. The most common dermatologic toxicities include maculopapular, erythematous rash, or pruritus. Vitiligo can also be seen and is considered a positive prognostic factor in patients with melanoma, as it signals an immune attack on melanocytes. Frequently, irAEs involved gastrointestinal tract. Grade 3/4 diarrhea/colitis was the most frequently observed serious adverse event in clinical trials. Compared to anti-CTLA-4, agents targeting the PD-1/PD-L1 pathway seem to be better tolerated, with a more favorable toxicity profile, emphasizing the distinct biologic features of the two pathways. One reason that could explain the reduced toxicity could be that the PD1/PD-L1 checkpoint interaction takes place peripherally, i.e., at the tumor site, whereas the CTLA4/B7 interaction occurs mostly centrally, i.e., in the lymphoid organs [53]. Most of the toxicity associated with anti-PD-1/PD-L1 was immune related, as well as with anti-CTLA-4 therapy [54]. The most frequent adverse events recorded, regardless of causality, were fatigue, decreased appetite, diarrhea, nausea, dyspnea, constipation, vomiting, rash, pyrexia, and headache [54]. The grade 3/4 adverse event rate was 14% in patients receiving nivolumab. Interestingly, one unique and potentially life-threatening toxicity for these agents is pneumonitis, which occurred in 3% of patients, but only 1%–3% developed a grade 3 or 4 pneumonitis [54, 55]. No clear relationship was reported between the incidence of this side effect and tumor type, the dose level, or the number of doses received. In the majority of cases, it was reversible with treatment discontinuation and/or glucocorticoid administration, but three patients died despite the use of infliximab and mycophenolate [54]. Mild infusion reactions were observed in patients receiving

anti-PD-L1 treatment, whereas severe adverse effects were infrequently noted [56]. Indeed, irAEs were observed in 39% of patients and included rash, hypothyroidism, hepatitis and, less frequently, sarcoidosis, diabetes mellitus, and myasthenia gravis. These adverse events were predominantly of grade 1 or 2 and were managed with treatment interruption or discontinuation. The grade 3/4 adverse event rate was 9% in patients receiving BMS-936559 [57] and was managed with glucocorticoids.

66.6 Future Perspectives

At present, knowledge about the interaction between carcinogenesis, immunity, and tumor biology is rapidly expanding. The first clinical data from new immune-mediated therapies in breast cancer are mainly promising for TNBC and HER2-positive breast cancer, possibly due to higher immunogenicity of these subtypes [16]. Biomarkers to select patients who will benefit from immunotherapy are needed, as shown by the large differences in immunogenicity between breast cancer subtypes. This is underlined by the prognostic value of TILs and PD-1 and PD-L1 in selected subgroups only. The highly dynamic nature of PD-L1 complicates the validation of this target as a relevant marker. Multiple clinical trials are now underway to evaluate breast cancer immunotherapy, including vaccines, adjuvants, and checkpoint blockade, alone or as multimodality therapy. The synergy of RT and immunotherapy represents a rising strategy with the potential to better target local irradiated/viable tumor cells and to give higher control of distant systemic disease. The future is bright, as the most effective immunotherapies may not only affect objective tumor responses acutely but also establish durable responses that promote the long-term control, and ultimately the cure, of breast cancer.

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Targeting PI3K/AKT/mTOR Pathway

67

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67.1 Introduction

Breast cancer is the principal cause of new cancer diagnosis and the second principal cause of cancer death in women in Europe and the United States [1, 2]. Our understanding of the biology of breast cancer has increased dramatically in the recent past, and progresses in molecular biology have uncovered a vast number of genomic aberrations. It is becoming more and more evident that a lot of these aberrations converge on a few key pathways involved in cancer cell signal transduction, including the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) cascade [3]. This pathway plays an important role in normal cellular physiology; the carcinogenic process involves and uses this same pathway to communicate constitutively active survival signals to the nucleus. Here we discuss why the PI3K/AKT/mTOR signaling pathway is potentially highly relevant to all three major subtypes of breast cancer (i.e., estrogen receptor (ER) positive/human epidermal growth factor receptor 2 (HER2) negative, HER2 positive, and ER negative/HER2 negative) and the rationale for inhibition of this pathway in breast cancer.

67.2 Biology of the PI3K/AKT/mTOR Pathway in Breast Cancer

Initially, it was determined that the transforming gene product of the Rous sarcoma virus phosphorylates phosphatidylinositol using PI3K as a catalyst [4]. Then, it was observed that the PI3K enzyme was a heterodimer consisting of a catalytic domain (p110) and a regulatory domain (p85). The PI3K pathway is crucial in controlling the normal physiological environment within the cell, and knockout of either α or β subunit of p110 leads to embryonic lethality in mice [5, 6]. Also, the PI3K pathway plays a paramount role in metabolism, growth, and survival of cancer cells in a number of human cancers, including breast cancer [7].

PI3K pathway signaling may be activated by G protein-coupled receptors (GPCRs) and via cell surface receptor tyrosine kinases (RTK) like epidermal growth factor receptor (EGFR, also called human epidermal growth factor receptor 1 or HER1), HER2, insulin-like growth factor 1 receptor (IGF-1R), and fibroblast growth factor receptor (FGFR) [3]. PI3K phosphorylates phosphatidylinositol diphosphate (PIP2) to form phosphatidylinositol triphosphate (PIP3) which eases the activation of the oncogene AKT. The tumor suppressor gene phosphatase and tensin homolog (PTEN) acts as the main negative regulator of the PI3K pathway by dephosphorylating PIP3 to PIP2. Inositol polyphosphate 4-phosphatase type II (INPP4B) is a newly described lipid phosphatase that switches PIP2 to phosphatidylinositol monophosphate (PIP) and could be a potential tumor suppressor, as well as a regulator of AKT activity [8, 9]. AKT is a serine-/threonine-specific protein kinase which phosphorylates effectors within cell survival, proliferation, and metabolic pathways [10]. Inhibition of tuberous sclerosis complex 2 (TSC2) by AKT stimulates the mammalian target of rapamycin (mTOR) leading to increased protein synthesis via its effector's eukaryotic translation initiation factor eIF4E-binding protein 1 (4EBP1) and p70S6 kinase (S6 K). Hence, AKT is a nodal point linking cell growth, apoptosis, and cellular metabolism. mTOR is an evolutionarily conserved serine/threonine kinase made of two distinct protein complexes, mTORC1 and mTORC2. Both complexes may be necessarily inhibited for optimally controlling cancer growth [11]. mTOR inhibitors like rapamycin and its analogs preferentially inhibit mTORC1. mTORC1 exerts negative feedback on the PI3K pathway (via S6K1 and IRS-1), and thus a selective blockade of mTORC1 alone may in fact improve cell growth by reflex activation of AKT mediated by mTORC2 [12]. Conversely, selective mTORC1 blockade using allosteric inhibitors is extremely efficient in cancer cell lines with PIK3CA mutations [13–15]. In addition, the BOLERO-2 study showed remarkable benefit of the mTORC1 inhibitor

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everolimus in combination with exemestane in postmenopausal hormone-receptor-positive advanced breast cancer patients who were resistant to anastrozole or letrozole [16]. Also, the PI3K pathway is important for normal glucose homeostasis, protein synthesis, and metabolism. Insulin and IGF-1 signaling through the PI3K pathway is a key mechanism for metabolism control, and the high incidence of its deregulation in breast and other cancers highlights the link between metabolism regulation and the oncogenic process [17]. PIK3CA mutations have been shown to activate the PI3K pathway and to be transforming in preclinical models [18, 19]. In humans, mutations of the PIK3CA gene are seen in about 25% of patients with breast cancer and are predominantly associated with the ER-positive and HER2-positive subtypes [20–23]. The PI3K pathway is often dysregulated in breast cancer [24]. PI3K pathway may become hyperactivated in breast cancer as a result of overexpression of upstream activators like HER2 or IGF-1, PTEN loss (which appears to predominate in triple-negative breast cancer [TNBC]), and gain-of-function mutations of the PIK3CA gene that codes for PI3K catalytic subunit (mostly seen in luminal and HER2-positive breast cancer) [25]. A report from the Cancer Genome Atlas Network involving 825 patients showed that there is substantial difference in the frequency of molecular alterations in the PI3K/AKT/mTOR pathway among the different clinical subtypes of cancer [23]. Another publication reported that PIK3CA point mutations were found in 27 of 79 ER-positive and 3 of 21 ER-negative BC tumor samples analyzed [26]. Loss of PTEN is a common means of activating PI3K signaling in human cancers [27]. However, PTEN mutation is only seen in about 3% of breast cancer cases, thus suggesting other mechanisms of regulation at the epigenetic, transcriptional, and posttranscriptional level, limiting the utility of simple protein or gene-expression-based assays to determine PTEN status [3, 26, 28–30]. Techniques for detection of PTEN loss are still evolving and not yet standardized. Epithelial cells have been demonstrated to be sensitive to decrease in PTEN abundance; in addition, microRNAs produced by the PTEN tumor suppressor gene and its pseudogene (called PTENP1) could play an important role in carcinogenesis [31]. PTEN-deficient tumors could be preferentially reliant on the p110β isoforms of PI3K [32]. Notably, some recent reports have failed to demonstrate a correlation between expression of PTEN and clinical outcome, both in early and advanced breast cancer [33, 34]. In a phase II study (n = 31 patients with pretreated breast cancer) using single-agent temsirolimus 25 mg weekly, the authors found no correlation between clinical benefit and PTEN loss or PIK3CA mutations [35]. Genetic alterations and constitutive activation of the PI3K pathway are likely to contribute to the pathogenesis of all three major breast cancer subtypes [28]. The classic example is that of HER2-positive breast cancer, where overexpression of the HER2 protein has long been associated with a poor prognosis [36]. HER2-amplified cell lines display profound sensitivity to PI3K, AKT, and/or mTORC1/mTORC2 inhibition, suggesting that HER2-overexpressing breast cancers are more dependent on dysfunctional PI3K/AKT/mTOR signaling [13, 15, 37–39]. While the prognostic influence of PIK3CA mutations is unclear in humans (particularly in ER-positive disease), increased sensitivity to tamoxifen in PIK3CA mutant versus wild-type cell lines has been observed [21, 40]. The biological mechanism for this is presently unidentified, and differences probably exist for the site-specific mutations. The increased sensitivity of the ER-positive/PI3K-mutant genotype to inhibitors of PI3K and mTOR in cell lines is interesting and raises the possibility that this group may represent an ideal population for clinical evaluation of these agents [41]. Reverse phase protein arrays (RPPA) studies are emerging as a useful tool for the functional analysis of kinases and steroid proteins. One study found that an 82-protein functional proteomic "fingerprint" was technically reproducible and able to classify 128 non-microdissected breast cancer surgical specimens into six prognostic subgroups that demonstrated a significant correlation with breast cancer subtypes identified by transcriptional profiling [42]. Creighton et al. used RPPA-based proteomic studies to show that the luminal B subtype of human breast cancer had hyperactivated PI3K signaling associated with lower ER levels. They were able to increase the ER level in cell lines by PI3K blockade and concluded that dual targeting of the PI3K and ER signaling pathways may be useful in a subset of patients with aggressive ER-positive breast cancers [43]. The potential use of PI3K/AKT/mTOR blockade in luminal cancers was reviewed recently [44]. The frequency of PIK3CA mutations in TNBC is quite low, but these tumors frequently display a loss of expression of PTEN (ranging from 35% to 50%) [23, 45–48]. Preclinical data suggest a strong contribution from the PI3K signaling pathways to TNBC tumor biology. Given the strong scientific rationale for PI3K pathway inhibition in breast cancer, results from clinical trials evaluating these agents are eagerly awaited by the breast cancer community.

67.3 PI3K/AKT/mTOR Pathway Inhibitors in Clinical Development

Traditionally, the earliest compounds used to block the PI3K pathway were mTOR inhibitors. The prototype mTOR inhibitor is rapamycin (also called sirolimus), a macrolide antibiotic first discovered in a soil sample from Rapa Nui (Easter Island). Rapamycin is an allosteric inhibitor of mTORC1 that has little effect on mTORC2 and seems to merely partially inhibit 4EBP1 [49]. Other analogs ("rapalogs") of rapamycin have been developed, including temsirolimus (CCI-779), everolimus (RAD001), and ridaforolimus (MK-8669/AP23573), which function via a kinase-independent mechanism. In breast cancer, single-agent temsirolimus showed promising preclinical activity [50]; however, its clinical

benefit has been modest [51, 52]. Wolff and colleagues reported results from a phase III trial in which 1112 patients with aromatase inhibitor-naive, hormone-receptor-positive advanced disease received letrozole plus oral temsirolimus/placebo [52]. There was no overall improvement in the primary endpoint progression-free survival (PFS), and the independent data monitoring committee recommended early closure of the study for futility at a preplanned interim analysis. In contrast, when everolimus plus exemestane and exemestane plus placebo were randomly assigned to 724 patients with hormone-receptor-positive advanced breast cancer with prior exposure to aromatase inhibitors in the phase III BOLERO-2 trial, the combination was found to have a significantly better PFS than exemestane alone (6.9 versus 2.8 months, HR 0.43, 95% CI 0.35–0.54, $p < 0.0001$), leading to the early termination of the trial at a preplanned interim analysis [16]. However, the effort to improve clinical efficacy by a more robust blockade of mTOR signaling has led to the development of newer mTOR inhibitors. These are ATP-competitive inhibitors of the catalytic activity of mTOR kinase and efficiently suppress both mTORC1 and mTORC2. The kinase domains of mTOR and PI3K are structurally similar, and this new generation of molecules potently inhibits them both, effectively making them PI3K/mTOR dual inhibitors [11]. Early attempts at PI3K inhibition with nonspecific compounds like quercetin, wortmannin, and LY294002 were useful in understanding the PI3K molecular pathway, but lack of specificity and poor pharmacology limited the clinical application of these agents. More specific and potent PI3K pathway inhibitors currently under development act either on multiple class I PI3K isoforms (e.g., buparlisib or BKM120, XL147, GDC-0941), have dual PI3K/mTOR inhibitor activity (e.g., dactolisib or BEZ235, BGT226, XL765), or inhibit AKT (e.g., MK-2206, GSK2141795) [53]. The clinical development of PI3K and mTOR inhibitors and their use in cancer have been reviewed elsewhere [54–57]. The Breast International Group (BIG), the German Breast Group (GBG), and the Spanish Breast Cancer Study Group (SOLTI) have launched a phase II randomized double-blind study of neoadjuvant trastuzumab versus trastuzumab plus BKM120 in combination with weekly paclitaxel in HER2-positive primary BC patients (NeoPHOEBE study, NCT01816594).

67.4 Toxicity

Since the PI3K/AKT/mTOR pathway plays central roles in normal human cell physiology, its blockade could result in severe or unexpected adverse events. The safety profile of these drugs must therefore be closely monitored over the long term. Results from clinical trials involving different single agents have already begun to reveal toxicity patterns and "class effects." For example, PI3K inhibitors may result in glucose intolerance. Given the role of PI3K in normal glucose metabolic control via IGF-1R, it is not astonishing that inhibition of this pathway leads to hyperinsulinemia and decreased glucose tolerance. Hyperglycemia induced by the pan-PI3K blocker PX-866 has been noted in animal models but is in general mild and reversible [58]. This effect is also apparent in human studies using the mTORC1 inhibitor temsirolimus, the class I selective PI3K inhibitor BKM120, and the AKT inhibitor MK-2206 [51]. The dual PI3K/mTOR inhibitor XL765 caused food-induced increase in plasma insulin, but not glucose, in an exposure-dependent fashion. However, the pan-PI3K inhibitor PX-866 did not show any changes in the insulin or glucose levels in a phase I study. The change from baseline for fasting glucose values may in fact be used as a surrogate pharmacodynamic marker of activity of drugs inhibiting the PI3K/AKT/mTOR pathway. Schedule-dependent, noninfectious pneumonitis is a recognized adverse effect of oral everolimus. In a phase II study, pneumonitis was seen in 46% of patients receiving 10 mg daily and 19% of patients receiving 70 mg weekly everolimus. Although it may initially present as asymptomatic radiological signs or with cough, it may be severe and dose limiting. It is not clear whether pneumonitis is a class- or pathway-specific toxicity. Management may require close monitoring of patients and effective and early intervention through dose modifications, interruptions, or supportive interventions. Apart from pneumonitis, the long-term use of mTOR inhibitors in renal transplant recipients has been associated with infection, proteinuria, edema, dermal eruption, and hyperlipidemia. In the BOLERO-2 study, the most common grade 3 or 4 adverse events included stomatitis (8% in the everolimus-plus-exemestane group versus 1% in the placebo-plus-exemestane group), anemia (6% vs. <1%), dyspnea (4% vs. 1%), hyperglycemia (4% vs. <1%), fatigue (4% vs. 1%), and pneumonitis (3% vs. 0%) [16]. Mood disorders have been associated with the use of BKM120 [59]. mTOR-associated enteritis has been reported in two patients enrolled in a phase II clinical trial of everolimus plus rituximab in advanced B-cell lymphoma and one patient on a phase II study of temsirolimus and bevacizumab for metastatic renal cell carcinoma [60]. Fatigue, infection, and mucositis were the main non-hematological toxicities in 47 patients with HER2-positive metastatic breast cancer receiving everolimus plus trastuzumab [61].

67.5 Future Perspectives

Prognosis of patients with breast cancer has improved significantly over the past few decades, mainly as a result of routine screening for breast cancer, improvements in surgery, radiation techniques, and systemic therapy. The only remarkable success of modern targeted therapy in improving breast

cancer outcome has been the advent of anti-HER2 agents. Although several genomic aberrations are seen in HER2-positive breast cancers, yet these tumors remain critically dependent on HER2 signaling for survival and growth, and anti-HER2 therapies exploit this phenomenon of "oncogene addiction" to deliver significant clinical benefit [62]. Similar improvement in clinical outcome by blockade of other targets is proving to be notoriously difficult to replicate, mainly because of cross talk and redundant signaling pathways [63]. Thus there is a need to characterize in greater detail the biology of the canonical PI3K/AKT cascade, one of the most frequently dysregulated signal transduction pathway in breast cancer. This will help identify critical dependencies on particular components and escape mechanisms. Furthermore, we need to understand more accurately the cross talk between the PI3K/AKT/mTOR pathway and other pathways. As most solid tumors are the result of multiple accumulated genetic aberrations that cause perturbations in several signaling pathways, it seems improbable that targeting any one of these anomalous pathways will lead to sustained tumor response. Hence, future strategies are likely to use the combination of two or even more targeted agents in order to avoid or significantly slow the traffic of signals down these pathways and thus achieve optimal and sustained clinical benefit. In general, targeted agents are very expensive, and the simultaneous use of multiple such drugs is likely to impose a significant financial burden on the healthcare system. In recent decades, the rational design of molecules against these targets has dominated the oncology drug development process, replacing previous methods of empirical screening of drug libraries. There is a need to further refine and improve the drug-designing process, in order to produce specific and potent targeted agents with fewer off-target effects. Once suitable candidates are identified, collaboration between different pharmaceutical companies will be necessary for optimal development of rational drug combinations. Targeted drugs need to be tested in smartly designed clinical trials incorporating optimal endpoints and innovative statistical design in order to minimize drug development "costs"—money, time, and number of patients required [64]. The optimal duration of targeted therapy and how to best incorporate it into standard treatment protocols also need to be determined [65]. Since the effects of some targeted drugs may be modest, and thus obscured by high tumor burden or development of resistance, it is likely that the neoadjuvant setting, including presurgical or window-of-opportunity design, may be a better paradigm for testing these new molecules as compared to the metastatic setting [66, 67]. The neoadjuvant setting may also provide an ideal opportunity to identify predictive and prognostic biomarkers, including imaging biomarkers. In future randomized trials, molecular screening could be used to select patients with specific genomic features and could yield substantially improved clinical response rates to targeted agents than patient selection based on traditional histopathological and clinical criteria [68]. Additionally, the combined use of biomarkers such as genotype (e.g., PIK3CA mutation), gene signatures, and sequencing-based approaches may help better define a responsive population [9, 21, 69, 70]. The promise of personalized oncology remains substantially unfulfilled to date. While there has been some progress in tailoring therapies according to breast cancer biomarkers (e.g., we have moved from viewing breast cancer as a single monolithic disease a couple of decades ago to two entities based on hormonal status to four clinicopathological entities currently—luminal A, luminal B, HER2-positive, and TNBC), truly personalizing therapy according to the complex molecular features of the individual patients is not yet a reality [71]. Despite the early hopes raised by microarray-based technologies, gene signatures still remain largely a research tool and with some exceptions like Oncotype DX and MammaPrint are not widely used in the clinic [72–74]. A systems biology approach to comprehensively assess DNA, RNA, protein, and metabolites could help identify key breast cancer molecular drivers and biomarkers [75, 76]. The huge amounts of data generated by sequencing and other "-omics" technologies present a formidable challenge—in terms of storage, handling, analysis, interpretation, and application. Strong technologies for dynamic in vivo assessment of response using functional imaging biomarkers or receptor imaging with newer PET radiotracers are needed to allow real-time readout of effects of individual targeted drugs. Significant efforts are already underway; examples include the development of zirconium-labeled trastuzumab, copper-labeled DOTA-trastuzumab, carbon-labeled GSK1120212, and carbon-labeled GSK2118436 (NCT01081600, NCT01093612, NCT01387204, and NCT01340833, respectively) [77]. In the future, this may allow clinicians to quickly adapt targeted therapy combinations based on tumor response and thus deliver truly personalized care to breast cancer patients.

Conclusions

Advances in molecular research have resulted in an improved understanding of breast cancer biology. Several targets of critical importance to breast cancer cells have been identified, such as ER, HER2, other RTKs, and components of the PI3K/AKT/mTOR pathway. As we unravel the molecular circuits of breast cancer in greater detail, we have begun to recognize the terrific complexity of carcinogenic mechanisms. Based on strong preclinical rationale, it is logical to envisage that the inhibition of the PI3K/AKT/mTOR pathway in selected breast cancer patients may lead to clinically significant benefit; however, this hypothesis needs to be tested further in clinical trials. Successful development of combinations will require determining the preferred

drug targets, doses and schedules, and supportive interventions that maximize therapeutic index (i.e., effective inhibition of target pathways in tumors for maximal anticancer effects with acceptable normal tissue sparing). Several such clinical trials are underway and results are eagerly awaited. By using biomarker-driven patient selection, serial pharmacodynamic evaluation, optimal treatment schedules, and rational combinations of therapies, the modern generation of cancer researchers and clinicians should be able to meet the great challenge of developing more effective breast cancer therapies in the near future.

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Targeting Genome Instability and DNA Repair

68

Marzia Locatelli and Giuseppe Curigliano

68.1 Introduction

Genomic instability is a characteristic of most human cancers and plays critical roles in both cancer development and progression.

Genomic stability is dependent on faithful DNA repair and chromosome segregation during cell division [1].

To maintain genomic integrity, eukaryotes have evolved a system called the DNA damage response (DDR). DDR is a complex signal transduction pathway that allows cells to sense DNA damage and transduce this information to the cell to arrange the appropriate cellular responses to DNA damage [2, 3]. The failure to respond to DNA damage is a characteristic associated with genomic instability. This instability can manifest itself genetically on several different levels, ranging from simple DNA sequence changes to structural and numerical abnormalities at the chromosomal level. During S phase, the centrosome and genomic material are replicated concurrently, and replication errors are repaired prior to mitotic entry. During mitosis, equal segregation of chromosomes requires a bipolar mitotic spindle, telomeric preservation, and completion of the spindle assembly checkpoint. Ectopic amplification of centrosomes, telomerase dysfunction, and failure of the spindle assembly checkpoint may result in aborted mitosis. The majority of cancers exhibit chromosomal instability (CIN), which refers to the high rate by which chromosome structure and number changes over time in cancer cells compared with normal cells [4]. Although CIN is the major form of genomic instability in human cancers, other forms of genomic instability have also been described. These include accumulation of DNA base mutations and microsatellite instability (MIN), a form of genomic instability that is characterized by the expansion or contraction of the number of oligonucleotide repeats present in microsatellite sequences [4–6], and forms of genomic instability that are characterized by increased frequencies of base pair mutations [7].

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68.2 Hereditary Versus Sporadic Cancers

Familial breast cancer (BC) accounts for approximately 5–10% of BC cases. The most prevalent mutations leading to hereditary breast and ovarian cancer affect the homologous recombination (HR) genes BRCA1 and BRCA2. Heterozygous individuals carrying mutations of the BRCA1 or BRCA2 genes have a 40–80% risk of developing BC [8].

Patients (pts) with BRCA2 mutations have increased incidence of male breast, pancreas, and prostate cancer [3]. Tumors with BRCA1 or BRCA2 mutations are significantly associated with low level of 53BP1, indicating that 53BP1 mutation might confer a survival advantage in the absence of BRCA1 and BRCA2 [9]. Moreover, mutations in three additional HR genes, BACH1, PALB2, and RAD51C, have been identified in approximately 3% of familial BC pts and have been associated with a twofold increased risk of BC [10]. Mutations of CHK2, ATM, NBS1, and RAD50 have also been associated with a doubled risk of BC, indicating the importance of the ATM pathway, together with HR, in preventing BC formation. In hereditary cancers that are characterized by the presence of CIN, the genomic instability can also be attributed to mutations in DNA repair genes. The identification of mutations in DNA repair genes in hereditary cancers provides strong support for the *mutator hypothesis*, which states that genomic instability is present in precancerous lesions and drives tumor initiation by increasing the spontaneous mutation rate [4, 11, 12]. According to mutator hypothesis, the genomic instability in precancerous lesions results from mutations in caretaker genes, that is, genes that primarily function to maintain genomic stability [4, 11, 12]. Indeed, in inherited cancers, germline mutations targeting DNA repair genes are

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present in every cell of the patient's body. Thus, a single event—loss of the remaining wild-type allele—would lead to genomic instability and drive tumor development, as predicted by the mutator hypothesis. The classical caretaker genes are DNA repair genes and mitotic checkpoint genes [4]. Germline mutations in caretaker genes can explain the presence of genomic instability in inherited cancers. However, efforts to identify caretaker genes, the inactivation of which leads to genomic instability in sporadic (nonhereditary) cancers, have met with limited success [4, 13]. Thus, unlike hereditary cancers, the molecular basis of genomic instability in sporadic cancers remains unclear. A second hypothesis could explain the presence of CIN in sporadic cancers. That is *the oncogene-induced DNA replication stress model for cancer development* [14–18]. According to the second model, CIN in sporadic cancers results from the oncogene-induced collapse of DNA replication forks, which in turn leads to DNA double-strand breaks (DSBs) and genomic instability [4].

68.3 Cellular Mechanisms that Prevent or Promote Genomic Instability

68.3.1 Telomere Damage

Telomeres, which are located at the ends of each chromosome, consist of approximately 5–10 kbp of specialized, tandem repeat, noncoding DNA complexed with a variety of telomere-associated proteins [1, 19, 20]. These elements create a protective cap that prevents the recognition of the chromosomal termini as DSBs and their consequent aberrant repair via nonhomologous end joining (NHEJ) or HR [1, 21–24]. Due to the inability of DNA polymerase to fully replicate the ends of linear DNA molecules, in the absence of compensatory mechanisms, telomeric DNA is lost at the rate of approximately 100 base pairs (bp) per telomere per cell division [1, 25–27]. In normal somatic cells, this telomere erosion is used by the cell to monitor its division history, with moderate telomere shortening triggering either irreversible cell-cycle arrest, termed replicative senescence, or apoptosis [1]. This block to continued proliferation is thought to have evolved to prevent the development of cancer in long-lived organisms by restricting the uncontrolled outgrowth of transformed cell clones and also by preventing further telomere erosion which would accompany such abnormal growth and eventually destabilize the telomeres leading to CIN [1, 25, 28].

68.3.2 Centrosomes

Centrosome amplification, the presence of greater than two centrosomes during mitosis, is a common characteristic of most solid and hematological tumors that may induce multipolar mitoses, chromosome missegregation, and subsequent genetic imbalances that promote tumorigenesis [1, 29].

The centrosome is the primary microtubule organizing center in dividing mammalian cells [1]. The centrosome is duplicated in a semiconservative fashion with one daughter centriole formed next to a preexisting mother centriole, and this process only occurs once in every cell cycle [1, 30, 31].

Centrosome amplification arises from many different mechanisms, including centrosome over duplication [1, 31, 32], de novo assembly [1, 33], and mitotic failure downstream from mono- [34] or multipolar division [35]. Given that centrosome clustering may be advantageous for cancer cell survival, this process may be an attractive and specific therapeutic target [36–38]. Bipolar chromosome attachment during mitosis is ensured by a quality control mechanism known as the spindle assembly checkpoint [1]. The assembly checkpoint relies upon kinase signaling to delay cell-cycle progression and correct attachment errors. Aurora kinase B, for example, detects misattached chromosomes [1], and overexpression of the kinase is sufficient to disrupt the checkpoint and promote tetraploidy [1]. Moreover, mutations or expression changes in other checkpoint gene products may compromise the checkpoint and favor tumorigenesis [39].

68.3.3 DNA Methylation and Chromatin Remodeling

A vast array of epigenetic mechanisms contribute to the genomic instability in cancer cells [40]. One of them is the DNA methylation, which consists of the addition of a methyl group at the carbon 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring [41]. Most cytosine methylation occurs in the context of cytosine-phosphate-guanine (CpG) dinucleotides and occurs via a group of DNA methyltransferase enzymes resulting in silencing of gene transcription [1]. A prominent example is the aberrant methylation of CpG islands in the promoter regions of DNA mismatch repair (MMR) genes that result in cancer cells with a "mutator phenotype" [1, 42]. In addition to DNA methylation, histone molecules that form the primary protein component of chromatin also regulate genome stability as well as gene transcription [43]. A number of posttranslational modifications such as acetylation, deacetylation, methylation, phosphorylation, and ubiquitination have been identified that alter the function of histones [1]. Various combinations of these posttranslational histone modifications have been hypothesized to form a "histone code" that dictate distinct chromatin structures that can affect genome stability pathways and transcription [1]. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.

In addition, histone acetylation can affect DNA repair. Similarly, histone ubiquitination can also modify DNA repair capacity [1, 44]. Finally, histone phosphorylation is an early event following DNA damage and required for efficient DNA repair [1].

68.3.4 Mitochondrial DNA Alteration in Human Cancers

Mitochondria are the key component of the oxidative phosphorylation system to generate cellular adenosine triphosphate. Mitochondrial genetic reprogramming and energy balance within cancer cells play a pivotal role in tumorigenesis [1]. Most human cells contain hundreds of nearly identical copies of mt-DNA, which are maternally inherited. A substantial number of studies identified somatic mt-DNA mutations involving coding and noncoding mt-DNA regions in various cancers [1].

Table 68.1 DNA lesions generated by endogenous and exogenous DNA damage [3]

Exogenous DNA damage	Dose exposure (mSV)	DNA lesions generated
Peak hr. sunlight	–	Pyrimidine dimers (6-4) photoproducts
Cigarette smoke	–	DSBs
Chest-X-ray	0.02	DSBs
Mammography	0.4	DSBs
Body CT scan	7	DSBs
Tumor PET scan	10	DSBs
Airline travel	0.005/h	DSBs
Endogenous DNA damage	*Dose lesions generated*	*Number lesions/cell/day*
Depurination	AP site	10,000
Cytosine deamination	Base transition	100–500 s
SAM-induced methylation	3 meA	600
	7 meA	4000
	O^6 meG	10–30
Oxidation	8oxoG	400–1500

68.4 DNA Repair Pathways

Repeated exposure to both exogenous and endogenous insults challenges the integrity of cellular genomic material. To maintain genomic integrity, DNA must be protected from damage induced by environmental agents or generated spontaneously during DNA metabolism.

Environmental DNA damage can be produced by physical or chemical sources. For example, the ultraviolet (UV) component of sunlight can cause up to 1 × 105 DNA lesions per cell per day, many of which are pyrimidine dimers. If left unrepaired, dimers that contain cytosine residues are prone to deamination, which can ultimately result in cytosine being replaced with thymine in the DNA sequence. Likewise, ionizing radiation (e.g., from sunlight or cosmic radiation) can cause single-strand breaks (SSBs) and DSBs in the DNA double helix backbone. If misrepaired—for example, the inaccurate rejoining of broken DNA ends at DSBs—these breaks can induce mutations and lead to widespread structural rearrangement of the genome [45]. Table 68.1 [46, 47] showed environmental agents that cause DNA damage and mutations.

Spontaneous DNA alterations can be due to dNTP misincorporation during DNA replication, interconversion between DNA bases caused by deamination, loss of DNA bases following DNA depurination, and modification of DNA bases by alkylation. Additionally, DNA breaks and oxidized DNA bases can be generated by reactive oxygen species (ROS) derived from normal cellular metabolism.

Organisms respond to chromosomal insults by activating a complex damage response pathway. This pathway regulates known responses such as cell-cycle arrest and apoptosis (programmed cell death) and has been shown to control additional processes including direct activation of DNA repair mechanisms. Most of the subtle changes to DNA, such as oxidative lesions, alkylation products, and SSBs, are repaired through a series of mechanisms that is termed base excision repair (BER). In BER, damaged bases are first removed from the double helix, and the "injured" section of the DNA backbone is then excised and replaced with newly synthesized DNA [48]. Key to this process are members of the poly(ADP-ribose) polymerase (PARP) family. The PARP family has 16 members, but only PARP1 and PARP2 have been implicated in the DDR [49]. PARP1 and PARP2 are activated by SSBs and DSBs and catalyze the addition of poly (ADP-ribose) chains on proteins to recruit DDR factors to chromatin at breaks [3]. Mispaired DNA bases are replaced with correct bases by MMR [50]. In addition to BER, the pool of deoxynucleotides (deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dTTP), deoxyguanosine triphosphate (dGTP), and deoxycytidine triphosphate (dCTP)) that provide the building blocks of DNA can be chemically modified before they are incorporated into the double helix. The nucleotide pool is, therefore, continually "sanitized" by enzymes such as nudix-type motif 5 (NUDT5). Whereas small base adducts are repaired by BER, some of the bulkier single-strand lesions that distort the DNA helical structure, such as those caused by ultraviolet light, are processed by nucleotide excision repair (NER) through the removal of an oligonucleotide of approximately 30 bp containing the damaged bases. NER is often subclassified into transcription-coupled NER, which occurs where the lesion blocks and is detected by elongating RNA polymerase, and global-genome NER, in which the lesion is detected not as part of a blocked transcription process but because it disrupts

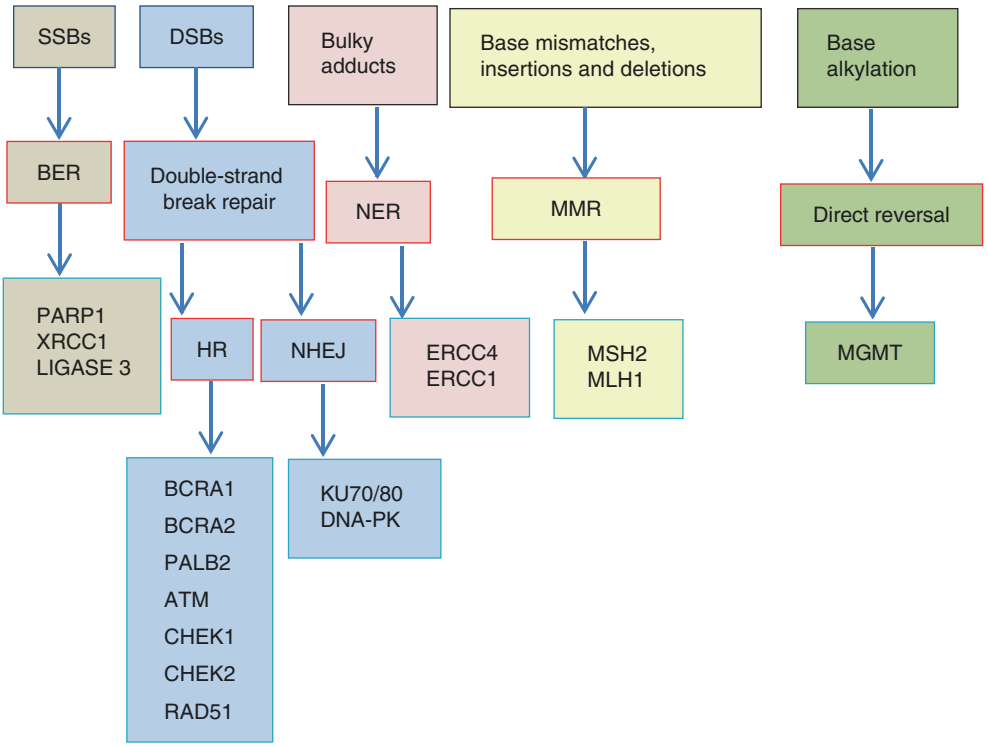

Fig. 68.1 DNA Repair Mechanisms maintain genomic stability. *SSBS* single-strand break, *DSBs* double-strand break, *HR* homologous recombination, *NHEJ* nonhomologous and joining, *MMR* mismatch repair

base pairing and distorts the DNA helix. Although these processes detect lesions using different mechanisms, they repair them in a similar way: DNA surrounding the lesion is excised and then replaced using the normal DNA replication machinery. Excision repair cross-complementing protein 1 (ERCC1) is key to this excision step. The major mechanisms that cope with DSBs are HR [51] and NHEJ [52]. HR acts mainly in the S and G2 phases of the cell cycle and is a conservative process in that it tends to restore the original DNA sequence to the site of damage. Part of the DNA sequence around the DSB is removed (known as resection), and the DNA sequence on a homologous sister chromatid is used as a template for the synthesis of new DNA at the DSB site. Crucial proteins involved in mediating HR include those encoded by the BRCA1, BRCA2, RAD51, and PALB2 genes. In contrast to HR, NHEJ occurs throughout the cell cycle. Rather than using a homologous DNA sequence to guide DNA repair, NHEJ mediates repair by directly ligating the ends of a DSB together. Sometimes this process can cause the deletion or mutation of DNA sequences at or around the DSB site. Therefore, compared with HR, NHEJ, although mechanistically simpler, can often be mutagenic.

SSBs repaired by single-strand break repair (SSBR), whereas DSBs are processed either by NHEJ or HR [3]. DNA repair is carried out by a plethora of enzymatic activities that chemically modify DNA to repair DNA damage, including nucleases, helicases, polymerases, topoisomerases, recombinases, ligases, glycosylases, demethylases, kinases, and phosphatases.

In summary DDR can be divided into a series of distinct, but functionally interwoven, pathways, which are defined largely by the type of DNA lesion they process (Fig. 68.1). DDR pathways encompass a similar set of tightly coordinated processes: namely, the detection of DNA damage, the accumulation of DNA repair factors at the site of damage, and finally the physical repair of the lesion.

MMR [50] is crucial to the DDR. Key to the process of MMR are proteins encoded by the mutS and mutL homologue genes, such as MSH2 and MLH1.

Finally, translesion synthesis and template switching allow DNA to continue to replicate in the presence of DNA lesions that would otherwise halt the process. Translesion synthesis and template switching are therefore usually considered to be part of the DDR. In translesion synthesis, relatively high-fidelity DNA replication polymerases are transiently replaced with low-fidelity "translesion" polymerases that are able to synthesize DNA using a template strand encompassing a DNA lesion. Once the replication fork passes the site of the lesion, the low-fidelity DNA polymerases are normally replaced with the usual high-fidelity enzyme, which allows DNA synthesis to continue as normal. In template switching, the DNA lesion is bypassed at the replication fork by simply leaving a gap in DNA synthesis opposite the lesion. After the lesion has passed the replication

fork, the single-strand gap is repaired using template DNA on a sister chromatid, similar to the process used during HR.

Although sometimes considered distinct from the DDR, the mechanisms that control the integrity of telomeric DNA at the end of each human chromosome also act as a barrier against genomic instability and mutation [53].

The core DDR machinery does not work alone but is coordinated with a set of complementary mechanisms that are also crucial to maintaining the integrity of the genome. For example, chromatin-remodeling proteins allow the DNA repair apparatus to gain access to the damaged DNA [54]. DDR core components interact with the cell-cycle checkpoint and chromosome segregation machinery. These interactions allow DNA repair to occur before mitosis takes place and ensure that the correct complement of genetic material is passed on to daughter cells [55].

68.5 Therapeutic Targeting of Genomic Instability in BC

When as CIN, and as changes to the structure of DNA, such as nucleotide substitutions, insertions, and deletions, they occur in crucial "driver" genes (of which there are probably fewer than ten per tumor), these mutations can alter cell behavior, confer a selective advantage, and drive the development of the disease. Importantly, these mutations can also influence how the tumor will respond to therapy. Alongside key driver mutations, emerging data from cancer genome sequencing suggests that a typical tumor may contain many thousands of other genetic changes. These "passenger" mutations do not contribute directly to the disease but are probably collateral damage from exposure to various environmental factors or defects in the molecular mechanisms that maintain the integrity of the genome. DNA damage causes cell-cycle arrest and cell death either directly or following DNA replication during the S phase of the cell cycle. Cellular attempts to replicate damaged DNA can cause increased cell killing, thus making DNA-damaging treatments more toxic to replicating cells than to non-replicating cells. However, the toxicity of DNA-damaging drugs can be reduced by the activities of several DNA repair pathways that remove lesions before they become toxic. The efficacy of DNA damage-based cancer therapy can thus be modulated by DNA repair pathways. In addition, some of these pathways are inactivated in some cancer types. These two features make DNA repair mechanisms a promising target for novel cancer treatments. Increasing knowledge of DNA repair permits rational combination of cytotoxic agents and inhibitors of DNA repair to enhance tumor cell killing. Thus, DNA repair inhibitors can be used in combination with a DNA-damaging anticancer agent. This will increase the efficiency of the cancer treatment by inhibiting DNA repair-mediated removal of toxic DNA lesions.

Moreover DNA repair inhibitors can be used as monotherapy to selectively kill cancer cells with a defect in the DNA damage response or DNA repair. Synthetic lethal interactions between a tumor defect and DNA repair pathway can be used to identify novel treatment strategies.

High levels of DNA damage cause cell-cycle arrest and cell death. Furthermore, DNA lesions that persist into the S phase of the cell cycle can obstruct replication fork progression, resulting in the formation of replication-associated DSBs. Evidence is also building that the DDR is not only invoked but also dysfunctional at an early stage in the development of neoplasia. Markers of DSBs, such as nuclear γH2AX foci (a histone phosphorylation event that occurs on chromatin surrounding a DSB), are markedly elevated in some precancerous lesions [14, 17]. The activation of oncogenes such as MYC and RAS stimulates the firing of multiple replication forks as part of a proliferative program. These forks rapidly stall, collapse, and form DSBs because they exhaust the available dNTP pool or because multiple forks collide on the same chromosome. Regardless of the mechanism, stalled and collapsed forks normally invoke the DDR and cell-cycle checkpoints that enable DNA lesions to be repaired before mitosis takes place. For precancerous lesions to progress to mature tumors, it is thought that critical DSB signal transduction and cell-cycle checkpoint proteins, such as ataxia telangiectasia (ATM) and ATM-Rad3 related (ATR), and the master "gatekeeper" protein p53 become inactivated. With these DDR components rendered dysfunctional, collapsed forks are not effectively repaired, and cells proceed through the cell cycle with DNA lesions intact, increasing the chance of mutagenesis [14, 17].

Common types of DNA damage that interfere with replication fork progression are chemical modifications (adducts) of DNA bases, which are created by reactive drugs that covalently bind DNA either directly or after being metabolized in the body. These *alkylating agents* are grouped in two categories: *monofunctional alkylating agents* with one active moiety that modifies single bases and *bifunctional alkylating agents* that have two reactive sites and cross-link DNA with proteins or, alternatively, cross-link two DNA bases within the same DNA strand (intrastrand cross-links) or on opposite DNA strands (interstrand cross-links). Interstrand cross-links pose a severe block to replication forks.

Despite the adverse side effects caused by alkylating agents on the bone marrow and other normal tissues, drugs such as cyclophosphamide, ifosfamide, chlorambucil, melphalan, and dacarbazine remain some of the most commonly prescribed chemotherapies in adults and children with various solid and hematological malignancies, particularly in combination with anthracyclines and steroids in multi-agent

regimens. The repair of alkylated lesions is thought to be quick, with the majority of lesions probably being repaired within 1 h. If the lesions are removed before the initiation of replication, the efficiency of alkylating agents in killing the tumor is significantly reduced. Thus, modulation of DNA repair that clearly influences the efficacy of alkylating agents is often explained by increased expression and/or activity of DNA repair proteins.

Antimetabolites, such as 5-fluorouracil (5FU) and thiopurines, resemble nucleotides, nucleotide precursors, or cofactors required for nucleotide biosynthesis and act by inhibiting nucleotide metabolism pathways, thus depleting cells of dNTPs. They can also impair replication fork progression by becoming incorporated into the DNA [56].

An alternative approach of interfering with replication is to target specific DDR components. Topoisomerase inhibitors, such as irinotecan (a topoisomerase I inhibitor) and etoposide (a topoisomerase II inhibitor), could be considered as the first generation of DDR targeted agents [45]. Topoisomerases are a group of enzymes that resolve torsional strains imposed on the double helix during DNA transcription and replication. They induce transient DNA breaks to relax supercoiled DNA or allow DNA strands to pass through each other [57]. Etoposide and irinotecan that inhibit this function leave DNA breaks across the genome. Topoisomerase II poisons cause DSBs, and topoisomerase I poisons cause positive supercoils in advance of replication forks and replication-associated DSBs [57].

PARP inhibitors as targeted therapy: PARP inhibitors are the next generation of DDR inhibitors.

It has been reported that expression levels of DNA repair genes are frequently associated with chemotherapy sensitivity and prognosis in BC subtypes. The poly(ADP-ribose) polymerase-1 (PARP1), one of the best characterized nuclear enzymes of the 17-member PARP family, participates in the repair of DNA SSB via the base excision repair pathway.

PARP1 and PARP2 catalyze the polymerization of ADP-ribose moieties onto target proteins (PARsylation) using NAD^+ as a substrate, releasing nicotinamide in the process. This modification often modulates the conformation, stability, or activity of the target protein [45]. The best understood role of PARP1 is in SSBR, a form of BER. PARP1 initiates this process by detecting and binding SSBs through a zinc finger in the PARP protein. Catalytic activity of PARP1 results in the PARsylation of PARP1 itself and the PARsylation of a series of additional proteins, such as XRCC1 and the histone H1 and H2B; when PARP activity is inhibited, SSBR is compromised [45].

The PARP inhibitors have been shown a substantial efficacy for hereditary BRCA1/2-related and triple-negative BC (TNBC) therapy [58–60]. Meanwhile, there are reports demonstrating that PARP inhibitors might be also active in non-hereditary BC cells lacking mutations in BRCA1 or BRCA2 [60, 61]. From a historical perspective, PARP-1 inhibitors entered the arena as promising co-adjuvant components of standard chemo-and radiotherapy regimens. Later, the discovery that tumor cell lines bearing deficiencies or mutation in DNA-repair genes (e.g., BRCA1 or BRCA2) do not tolerate PARP-1 inhibition fuelled the application of PARP inhibitors as single agent therapies in breast and ovarian BRCA-mutated cancer settings. More recently, the discovery of new potential combinative synergisms (e.g., PI3K, NAMPT, and EFR inhibitors) as well as the broadening of "synthetic lethality" context (e.g., PTEN and ATM mutations, MSI colorectal cancer phenotypes and Ewing's sarcomas) in which the inhibition of PARP-1 can be therapeutically valuable has further raised interest in this target.

PARP inhibitors were designed to imitate the nicotinamide portion of NAD^+ with which they compete for the corresponding PARP-1 binding site. PARP inhibition probably works by allowing the persistence of spontaneously occurring SSBs or by inhibiting PARP release from a DNA lesion. Whichever is the case, both of these DNA lesion types could credibly stall and collapse replication forks, potentially creating lethal DSBs [45]. Recent data propose an indirect mechanism, according to which PARP1 activity would be dispensable for BER sheer execution and would be rather engaged to seize potentially detrimental SSB intermediates and to promote their resolution. Recently, PARP1 contribution to SSB repair has also been extended to MMR and NER. In normal cells, the effects of PARP inhibition are protected by HR, which repairs the resultant DSB. However, effective HR is reliant on functioning BRCA1 and BRCA2, so when these genes are defective—as they are in tumors of germline BRCA-mutant carriers—DSBs are left unrepaired, and potent PARP inhibitors can cause cell death. BRCA1 plays a role in both the G1/S and G2/M cell-cycle checkpoint regulation in response to DNA damage, again preserving genomic integrity. Moreover, the sensitivity to PARP inhibitors seems to be defined more by the BRCA genotype of a cancer cell than by its tissue of origin. Breast, ovarian, and prostate cancers with BRCA mutations all seem to be profoundly sensitive to these drugs.

As early as in 1980, Durkacz and colleagues used the still immature, low-potency PARP inhibitor 3-aminobenzamide (3-AB) to derail DNA damage repair and enhance the cytotoxicity of dimethyl sulfate, a DNA alkylating agent [62].

The first clinical trial in pts was initiated in 2003 and allowed safety, pharmacokinetic, and pharmacodynamic evaluation of the PARP inhibitor AG014699 (*rucaparib* [63]) in combination with temozolomide (TMZ), a DNA alkylator and methylator, in advanced solid tumors [64]. However, the subsequent phase II study in melanoma [65], as well as additional independent clinical trials, featured a common (albeit not universal) shortcoming of combinatorial strategies with PARP inhibitors, namely, enhanced toxicity. Myelotoxicity was the main dose-limiting concern in the face of variable

Table 68.2 PARP Inhibitors under investigation

PARP inhibitor	Cancer type
Veliparib	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
Niraparib (Nira, MK4827)	Ovarian cancer and BRCA+ breast cancer
Olaparib (Ola, AZD2281)	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
Iniparib (BSI-201)	Breast cancer, ovarian cancer, lung cancer, glioma, glioblastoma
Rucaparib (AG014699)	Breast and other solid tumors
BMN-673	Ovarian, breast, gastric, colorectal, and pancreatic tumors and a range of other solid tumors
CEP9722	Lymphoma, breast, ovarian cancer
E7016	Melanoma
AZD-2641	Solid tumors
INO-1001	Melanoma, breast cancer
E7449	Melanoma, breast cancer, ovarian, B-cell malignancies

response rates. The need to reduce the dosage of either chemotherapy or PARP inhibitor (or both) to overcome excessive toxicity raises obvious questions about the real contribution of PARP inactivation to combinatorial regimens.

Currently, almost eight PARP inhibitors are at different stages of clinical investigation, targeting several tumor types either as single agents or in combination (Table 68.2).

Veliparib (Veli, ABT-888) is a potent, oral inhibitor of PARP-1 and PARP-2 [66]. It is orally bioavailable and crosses the blood-brain barrier. Veli potentiated the cytotoxic effect of TMZ in several human tumor models. ABT-888 was investigated in an innovative phase 0 trial, the first such study in oncology [67]. The primary study endpoint was target modulation by the PARPi. There is an extensive clinical trial program associated with this agent with 32 ongoing clinical trials of Veli in combination with cytotoxics in ovarian, breast, colorectal, prostate, liver cancers, neurologic malignancies, and leukemias. In a phase II study [68], combined ABT-888 and TMZ is active in metastatic BC (MBC). Exploratory correlative studies including BRCA mutation analysis are underway to determine predictors of response. The dose and schedule of Veli suggest the clinical activity seen is not likely due to Veli alone but rather to the combination. Promising antitumor activity was observed in pts with BRCA mutations.

Olaparib (Ola, AZD2281) also inhibits PARP-1 and PARP-2 at nanomolar concentrations. Preclinical studies have largely concentrated on investigations of synthetic lethality in BRCA1 or BRCA2 defective models or combinations with platinum in these models. The first clinical study of PARP inhibition in BRCA-mutant cancers was with this agent. In this phase I study which enrolled 60 pts, Ola doses were escalated from 10 mg daily for 2 of every 3 weeks to 600 mg twice daily [69]. Olaparib is one of the most investigated PARP inhibitors through clinical trials either as monotherapy [70, 71] or in combination with other anticancer drugs [72–77]. There is general agreement that 400 mg b.i.d. is the maximum tolerable dose of Ola. At this dose, Ola exhibited an acceptable safety profile. Most common adverse effects reported are of Grade 1/2 type, such as procedural pain, nausea, and other gastrointestinal symptoms of mild to moderate intensity, and thus are manageable. An important outcome of combination phase I trials results is the general tolerance of Ola when given in combination with bevacizumab [74], cediranib [75] and liposomal doxorubicin [77]. Ola-paclitaxel combination against TNBC [76], as well as the Ola-CDDP combination against breast or ovarian cancer in pts carrying germline BRCA1/BRCA2 also report partial efficacy. In both studies, dose-limiting hematological toxicities were neutropenia and thrombocytopenia.

Five phase II trials were conducted with Ola alone. As with the phase I clinical trials for Ola, despite inherent differences in the study design, cancer types, patient variability, and evaluation protocols, important similarities are evident in the outcomes of these phase II clinical trials. A study in pts with confirmed BRCA1 or BRCA2 mutations and recurrent ovarian cancer [78] yielded the objective response rate (ORR) of 33% for Ola 400 mg b.i.d. In pts with BRCA1 or BRCA2 mutations and advanced BC, ORRs were significantly higher (41%) for the 400 mg dose [79]. In another study conducted at this dose level [80], TNBC pts with or without BRCA mutations failed to show any objective response (OR). Interestingly, in the same study, a very strong ORR of 41% was obtained for ovarian cancer pts with BRCA1 or BRCA2 mutations; pts without the BRCA1 or BRCA2 mutations also responded at a robust ORR of 11% [80]. In summary in phase II clinical studies, 40% of pts with breast or ovarian cancer with germline BRCA mutations had a favorable response to the drug. This is a particularly high response given that the pts in these trials had been heavily pretreated and had become resistant to a range of chemotherapies [45, 64].

INO-1001 is an isoindolinone derivative and is being developed for both oncological and cardiovascular indications. Preclinical studies demonstrate its protective effect in models of cardiac dysfunction and reversal of TMZ resistance in MMR-defective xenografts. This agent is being developed in oncology in melanoma and glioma and as a single agent in cancer for BRCA1- and BRCA2-deficient tumors. In phase I trials, INO-001was tested alone or in combination with TMZ [83]. Pharmacokinetic analyses indicate lack of interactions between TMZ with INO1001 and establish a “safe to administer” dose of the combination for further evaluation of the efficacy of INO1001 against

advanced melanoma. However, outcomes of some clinical trials are less encouraging.

CEP9722 in phase I trials was tested alone or in combination with TMZ [84]. These dose escalation phase I trials established what the authors call an "adequately tolerated" dose for these compounds. Thus, while no neutropenia and other hematological toxicities were noticed, dose-dependent PARP inhibition was also not observed, with only limited clinical activity.

Niraparib (Nira, MK4827) is a potent inhibitor of PARP-1 and PARP-2 that is currently in phase III clinical trials for ovarian cancer and BRCA+ BC. In a phase III, randomized, open-label, multicenter, controlled trial, Nira was compared versus physician's choice in previously treated, HER2-negative, germline BRCA mutation-positive BC pts. MK4827 (in a 2:1 ratio) is administered once daily continuously during a 21-day cycle. Physician's choice will be administered on a 21-day cycle. Health-related quality of life will be measured. The safety and tolerability will be assessed by clinical review of adverse events (AEs), physical examinations, electrocardiograms (ECGs), and safety laboratory values.

Iniparib (BSI-201) is an anticancer agent with PARP inhibitory activity in preclinical models. Although the full mechanism of its antitumor activity is still under investigation, iniparib enhances the antiproliferative and cytotoxic effects of carboplatin and gemcitabine in vitro models of TNBC. Phase I–Ib studies of iniparib alone and iniparib in combination with chemotherapy in pts with advanced solid tumors have shown iniparib to have mild toxicity, with no maximal dose reached in terms of side effects. O'Shaughnessy et al. [59], in a phase II trial, evaluate whether iniparib could potentiate the antitumor effects of gemcitabine and carboplatin with acceptable toxicity levels. A total of 123 pts were randomly assigned to receive gemcitabine (1000 mg per square meter of body-surface area) and carboplatin (at a dose equivalent to an area under the concentration-time curve of 2) on days 1 and 8—with or without iniparib (at a dose of 5.6 mg per kilogram of body weight) on days 1, 4, 8, and 11—every 21 days. Primary end points were the rate of clinical benefit (CB) (i.e., the rate of OR [complete or partial response] plus the rate of stable disease (SD) for ≥6 months) and safety. Additional end points included the ORR, progression-free survival (PFS), and overall survival (OS). The addition of iniparib to chemotherapy improved the CB and OS of pts with metastatic TNBC without significantly increased toxic effects. On the basis of these results, a phase III trial adequately powered to evaluate overall survival, and progression-free survival is being conducted.

In summary, there are many differences in the studies evaluating anticancer activity of PARP inhibitors used alone or in combination with one or more anticancer agents. While there are many differences in the studies, some common observations should be noted with particular emphasis on various enzymatic activities associated with this multi-domain group of proteins as it applies to developing new anticancer agents and/or regimens. Specifically, the discovery of activation of PARP-2 and PARP-3 by phosphorylated DNA ends mimicking substrates or intermediates in various DNA repair pathways is quite important. These observations shed new light on the molecular functions of different PARPs. Additionally, better understanding of the substrate specificity of individual members of the PARP family will allow researchers to further refine inhibitor chemistry and minimize adverse effects of drugs currently under evaluation. Another area of considerable potential for research and development of PARP inhibitors as first-line anticancer drugs is their application to personalized medicine. Targeted therapy is rapidly becoming a hallmark of a number of anticancer drugs.

Platinum chemotherapies: Cisplatin, carboplatin, and oxaliplatin have become three of the most commonly prescribed chemotherapeutic drugs used to treat solid cancers in pts [57]. Platinum resistance, either intrinsic or acquired during cyclical treatment, is a major clinical problem as additional agents that can be added to therapy in order to circumvent tumor resistance do not currently exist. Platinum chemotherapy is now being tested with PARP inhibition clinical trials. The rationale for combining PARP inhibition with platinum chemotherapy is based on preclinical observations that PARP inhibitors preferentially kill neoplastic cells and induce complete or partial regression of a wide variety of human tumor xenografts in nude mice treated with platinum chemotherapy [57]. For example, Veli has been shown to potentiate the regression of established tumors induced by cisplatin, carboplatin therapy in rodent orthotopic, and xenografts models [57]. However, the biological mechanisms of chemo-sensitization of cancer cells to platinum chemotherapy by PARP inhibition remain to be resolved.

Ionizing radiation and radiomimetic agents such as bleomycin cause replication-independent DSBs that can kill non-replicating cells. In addition, such treatments can also rapidly prevent DNA replication by activation of cell-cycle checkpoints to avoid formation of toxic DNA replication lesions [57].

Targeting microsatellite instability (MSI): MSI is a marker of defective MMR. The predictive value of MMR status as a marker of response to 5fluorouracil, irinotecan, and other drugs is still controversial. Two large retrospective analyses from several randomized trials confirmed the detrimental effect of a 5 fluorouracil-based adjuvant therapy in stage II colorectal patients [81–83], not applicable to stage III patients [84]. These latter authors, however, reported that MSI stage III tumors harboring genetic mutation in the MMR genes seem to benefit from the 5 fluorouracil adjuvant therapy. These data imply that molecular differences within the MSI subgroup influence the response to 5 fluorouracil. Combination therapy with methotrexate (MTX) and PARP inhibitors may be effective against tumors with MMR mutations. MTX elevates ROS and DSBs and the combination of

MMR mutation and PARP inhibition may attenuate repair and induce growth arrest or apoptosis [85–87].

Targeting gene expression of cell cycle and DNA repair components: Resveratrol, a phytoalexin produced by plants such as the Japanese knotweed, prevents hypermethylation of the BRCA1 promoter [88] and may be effective for TNBC or basal subtype BC. Other natural compounds, like genistein and lycopene, can alter DNA methylation of the glutathione S transferase p1 (GSTP1) tumor suppressor gene.

Targeting centrosome abnormalities: Griseofulvin, an antifungal drug that suppresses proliferation in tumor cells without affecting non-transformed cells, declusters centrosome, although the precise mechanisms behind the drug's action remain unknown [36]. In a similar fashion, depletion of a kinesin-like motor protein can selectively kill tumor cells with supernumerary centrosomes [36]. Finally, the PARP inhibitor PJ34 also declusters super numerary centrosomes without deleterious effects on spindle morphology, centrosome integrity, mitosis, or cell viability in normal cells [89–90].

Conclusion

Genomic instability plays a critical role in cancer initiation and progression. The fidelity of the genome is protected at every stage of the cell cycle. In cancer, the presence of aneuploid or tetraploid cells indicates the failure of one or many of these safety nets. The resultant genomic heterogeneity may offer the cancer "tissue" a selection advantage against standard of care and emerging therapies. Understanding these safety nets, and how they are bypassed in cancer cells, may highlight new and more specific mechanisms for cancer prevention or therapeutic attack. The therapeutic targeting of genomic instability may check and inhibit other enabling characteristic of tumors cells, such as replicative immortality, evasion of antigrowth signaling, and tumor promoting inflammation. To this end, vitamins, minerals, and antioxidants, such as vitamin B, vitamin D, carotenoids, and selenium, as well as nutraceuticals, such as resveratrol, have shown remarkable plasticity in elucidating antitumor responses. In addition to alleviating genomic instability, these compounds are known to inhibit proliferative signaling, attenuate oncogenic metabolism, and block inflammation.

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Targeting the CDK4/6 Pathway in Breast Cancer

69

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69.1 Introduction

Uncontrolled chronic proliferation is a fundamental hallmark of cancer [1]. In mammalian cells, the control over cell cycle entry and the progression from Gap 1 (G1) to Synthesis (S) is a particularly critical checkpoint. A key effector of this checkpoint is the retinoblastoma susceptibility gene product (Rb) which, like other Rb family members (such as p107 and p130), functions by binding and inactivating E2F transcription factors [2]. Rb activity is mainly regulated through phosphorylation by cyclin-dependent kinases (CDK), a group of serine/threonine kinases whose activity depends on binding of regulatory proteins known as cyclins. CDK4 and 6, which bind preferentially to D-type cyclins [3, 4], are the initial CDK to phosphorylate Rb, followed by other complexes such as cyclin E–CDK2. Fully phosphorylated Rb then induces the release of E2F transcription factors with consequent transcription of genes required for S-phase entry. CDK activity and progression of the cell cycle through G1-S checkpoint are negatively modulated by the universal CDK inhibitors of the Cip-Kip family, including p21Cip1 and p27Kip1, and the specific CDK4 and CDK6 inhibitors of the INK4 family, typified by p16 INK4a [5–9]. The p16 gene product inhibits formation of active D cyclin-CDK complexes through specific binding interactions with CDK4 or CDK6 that prevent D cyclin-CDK association [10–12] Fig. 69.1.

Homeostasis of adult healthy tissue requires that cells enter the cell cycle in a highly regulated manner. Tight regulation of the cyclin D-CDK4/6 axis is obtained by control of different steps including transcription of the Cyclin D1 gene, assembly of the Cyclin D1-CDK4/6 complex, and its nuclear transport and stability. All these processes are regulated via complex biochemical mechanisms whose description in detail is beyond the scope of this chapter. However, to appreciate the role of D-type cyclins in cancer, these should be viewed as major drivers of cell cycle progression under mitogenic stimuli [13]. Indeed, the CDK4/6 axis physiologically responds to a variety of extracellular stimuli including those activated by growth factors and receptors (e.g., RAS, mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR)), cytokines, cell adhesion machinery, and nuclear receptors (e.g., the estrogen receptor (ER)). Most of these stimuli also represent well-known oncogenic drivers in many cancer types, including breast cancer, thus highlighting one of the layers of involvement of the Cyclin D-CDK4/6 axis in cancer development, i.e., its role in mediating proliferation signals from oncogenic pathways.

An additional layer of involvement resides in the fact that genetic alterations, including mutations, amplification, and deletions in members of the Cyclin D-CDK4/6 axis, are frequently detected in many cancer types (recently reviewed in [14]). In physiologic conditions, some degree of functional redundancy exists among members of the Cyclin D1-CDK4/6 axis. This has been elegantly demonstrated by studies in transgenic mice where abrogation of the function of individual genes *Ccnd1*, *Cdk4/6*, and *Cdkn2a* (encoding for p16 protein), while resulting in focal developmental defects, still permitted the generation of viable mice. These observations generated the notion that individual members of this pathway are nonessential for the cell cycle itself [15, 16]. In contrast, as suggested by models of oncogene-induced cancer, members of the CyclinD-CDK4/6 axis might be essential for the development of some cancer types under specific oncogenic insult and might therefore represent unique therapeutic targets in these settings. As an example, studies using mouse tumor models where oncogenes such as Erbb2 and ras are under

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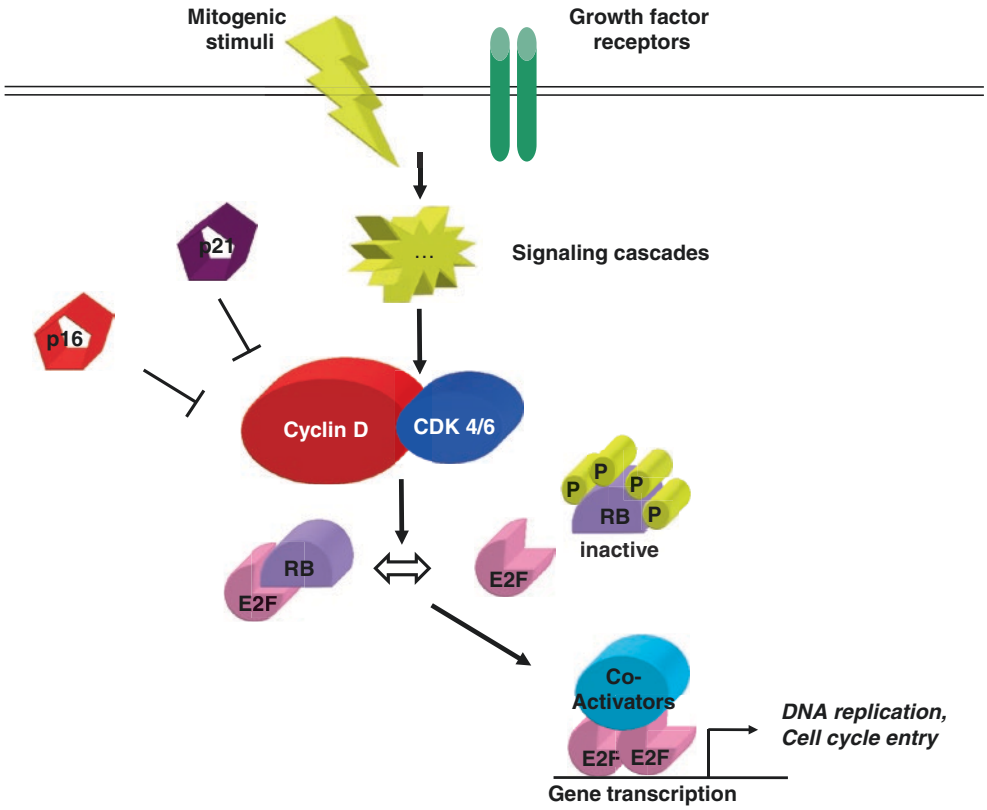

Fig. 69.1 Schematic representation of the CDK4/6 pathway

the control of the mouse mammary tumor virus (MMTV) promoter have indeed shown that breast tumor formation is impeded in the absence of CDK4 or CyclinD1 [17, 18]. However, the absence of cyclin D1 has no effect on breast tumor development induced by other oncogenes such as c-myc or Wnt-1 [19, 20]. Of note, the expression of neither CDK4 or Cyclin D1 is essential for mammary gland development [21]. These observations suggest that perturbations in the Cyclin D-CDK4/6 axis may represent crucial targets for cancer treatment in specific oncogenic contexts.

69.2 CDK4/6 Inhibitors as Anticancer Agents: Early Clinical Experiences and Phase I Studies

Given the central role of CDK in cancer, it is not surprising that several CDK inhibitors have been developed as potential antitumor drugs [22]. The first generation of CDK inhibitors included nonselective pan-CDK inhibitors, such as flavopiridol and roscovitine [14]. These compounds have been tested in numerous solid tumor types, but their clinical development has been hampered by their limited clinical activity and their unfavorable toxicity profile [14, 22]. Therefore, next-generation CDK inhibitors have subsequently been developed with specific activity toward individual CDK, including CDK4/6.

Three selective ATP-competitive, orally administered inhibitors of CDK4 and CDK6 have been synthesized, PD0332991 (palbociclib), LY2835219 (abemaciclib), and LEE011 (ribociclib), and are currently in different stages of clinical development.

Palbociclib, a highly specific inhibitor of CDK4 (IC50, 0.011 Mmol/L) and CDK6 (IC50, 0.016 Mmol/L), was the first compound to be developed and tested in clinical trials. It exerts G1 arrest with consequent antiproliferative activity in vitro and prevents tumor growth in vivo in a variety of Rb-positive tumor cells [23–27]. Palbociclib was first studied in two phase I dose-escalation trials in patients with advanced solid tumors or non-Hodgkin's lymphoma with histologically proven Rb tumor expression [28, 29]. In the first study, a total of 33 patients were enrolled across four cohorts receiving oral palbociclib at 100 mg ($n = 3$), 150 mg ($n = 4$), 200 mg ($n = 20$), and 225 mg ($n = 6$) daily doses [28]. A total of six patients experienced dose-limiting toxicities (DLT) consisting exclusively of myelosuppression (neutropenia with or without thrombocytopenia). Of these, two received a dose of 225 mg and four received 200 mg, which was identified as the maximum tolerated dose (MTD). Hematological toxicity in general was common. Grade 3 or 4 hematological adverse events consisted of lymphopenia (36%), neutropenia (24%), leukopenia (21%), thrombocytopenia (9%), and anemia seen in a single patient (3%). The most common non-hematological adverse events were

fatigue, nausea, and diarrhea. Treatment was generally well tolerated, and no patient discontinued treatment permanently because of treatment-related adverse events [28]. Of 31 patients evaluable for response, one patient with non-seminomatous germ cell testicular tumor had a partial response (PR), and nine patients (29%) experienced stable disease (SD). Stable disease was observed in four liposarcomas, one thyroid tumor, one melanoma, one cholangiosarcoma, and one angiomyxoma. Stable disease lasting ten cycles or more was observed in two patients with liposarcoma. In the second phase I trial, 41 patients were enrolled to receive palbociclib in six cohorts at doses ranging from 25 to 150 mg once daily on a 3 weeks on and 1 week off schedule [29]. DLT were observed in five patients (12%) at 75, 125, and 150 mg doses. MTD was 125 mg once daily. On the basis of these data, recommended phase II dose of palbociclib was 125 mg once daily. As in the other trial, hematological toxicity was common, and non-hematological adverse events consisted mostly of fatigue and nausea [29]. Of the 37 patients evaluable for response, none had PR and 13 (35%) maintained SD for at least two cycles, including 3 patients with liposarcoma and 2 with testicular tumors. Palbociclib subsequently moved into phase II and III clinical trials in different tumors, including breast cancer (see below).

As stated previously, hematological toxicities, especially neutropenia, were the most frequently reported adverse events for palbociclib. A recent work investigated the mechanism of palbociclib-induced bone marrow suppression and compared it to that induced by cytotoxic chemotherapeutic agents [30]. The authors demonstrated that, in contrast to chemotherapeutic agents which caused DNA damage and apoptotic cell death in human bone marrow mononuclear cells (hBMNCs), palbociclib did not induce senescence, with hBMNCs resuming proliferation following palbociclib withdrawal.

Abemaciclib is another orally available CDK4/6 inhibitor. In biochemical assays, abemaciclib inhibits CDK4/cyclinD1 and CDK6/cyclin D1 with IC50 = 2 nmol/L and 10 nmol/L, respectively, and inhibits Rb phosphorylation resulting in a G1 arrest and inhibition of proliferation in vitro and in vivo [31, 32]. Similar to palbociclib, its activity is specific for Rb-proficient cells [31]. In addition, preclinical studies in mice showed that abemaciclib crosses the blood-brain barrier and that brain levels are reached more efficiently at presumably lower doses and are potentially on target for a longer period of time than palbociclib [33]. The phase I trial of abemaciclib monotherapy included 132 patients with advanced solid malignancies. Results of this trial have so far been reported only in abstract form. Patients received abemaciclib 150–200 mg every 12 h continuously dosed, and a different toxicity profile was observed. Like palbociclib, neutropenia and fatigue were reported frequently; however, in contrast, the most common adverse events included gastrointestinal disorders such as diarrhea, nausea, and vomiting. In this trial, abemaciclib demonstrated activity in patients with breast cancer and non-small cell lung cancer (NSCLC) [34, 35].

Ribociclib is another CDK4/6 inhibitor. Its activity has been evaluated in vitro and in vivo. It has been shown to cause cell cycle arrest and cellular senescence in neuroblastoma-derived cell lines and growth delay in neuroblastoma xenografts [36] and to exert antiproliferative effects in CDK4-amplified human liposarcoma [37] and dermatofibrosarcoma protuberans in vitro and in vivo [38]. A total of 132 patients with advanced solid tumors and lymphomas were treated in a phase I trial with escalating doses of LEE011 on a 21-of-28-days or continuous schedule. DLT were observed in ten patients: neutropenia (3 pts), asymptomatic thrombocytopenia (2 pts), mucositis, pulmonary embolism, hyponatremia, QTcF prolongation (>500 ms), and increased creatinine (1 pt each). The MTD and the recommended phase II dose were declared as 900 and 600 mg/d on 21-of-28-d schedules, respectively. The most common study drug-related adverse events were neutropenia (40%), leukopenia (36%), nausea (35%), and fatigue (27%) [39].

69.3 CDK4/6 Inhibitors in Breast Cancer

In breast cancer, continuous cell proliferation is maintained by different mechanisms, including alterations involving the Rb pathway [40]. Cyclin D1 overexpression/amplification and CDK4 gains are frequent events, particularly in luminal breast cancer, while loss of RB1 and overexpression of $p16^{INK4A}$ occur mostly in basal-like carcinomas. Given the well-known lack of efficacy of CDK4/6 inhibitors in the context of RB1-negative models and the very high frequency of RB1 loss in basal-like and triple-negative breast cancers, the clinical development of CDK4/6 inhibitors has not been prioritized in these breast cancer subtypes. Conversely, given the high frequency of Cyclin D1 amplification or CDK4 gains in luminal breast cancers, most of the resources in terms of clinical development of CDK4/6 inhibitors have been invested in this subtype.

Accordingly, the majority of clinical trials have enrolled and are currently focusing on patients with hormone receptor (HR)-positive, HER2-negative tumors, often investigating associations of CDK4/6 inhibitors with endocrine therapy. However, new data on the role of CDK4/6 inhibitors in different breast cancer subtypes have accumulated in recent times. Here, we will review recent molecular and clinical advances on the role of CDK4/6 inhibitors in breast cancer therapy. In particular we will underline current progresses on the understanding of the role of CDK4/6 inhibitors in the context of the different breast cancer subtypes.

69.4 CDK4/6 Inhibitors in HR-Positive Breast Cancer

69.4.1 Preclinical Data

About three quarters of breast cancer cases express HRs (estrogen receptor—ER—and progesterone receptor (PR)) and are molecularly classified as luminal A or B. Among breast cancer subtypes, these tumors show the highest rate of cyclin D1 amplification and CDK4 gains and commonly retain Rb. The ER pathway and the Cyclin D-CDK4/6 axis are connected by extensive molecular cross talk. Indeed, ER directly controls Cyclin D1 expression at the transcriptional level [41], and estrogen modulation of E2F is critical for hormone regulation of the proliferative program of breast cancer cells [42]. However, the prognostic role of cyclin D1 amplification or overexpression in breast cancer is still controversial [43–47]. Recently, an integrated analysis of copy number and gene expression data in a large clinical annotated dataset of breast cancer patients has revealed a new, high-risk, ER-positive 11q13/14 cis-acting subgroup (IntClust 2). This subgroup includes both luminal A and B tumors and displays enrichment for Cyclin D1 amplification. However, other potential driver genes in the 11q13/14 amplicons are also amplified in this subgroup, including PAK1, RSF1, and EMSY, therefore suggesting that the particularly adverse outcome of this luminal population might be driven by a cassette of amplified genes rather than Cyclin D1 alone [48]. HR-positive breast cancers are preferentially treated with endocrine therapy, comprising drugs which target ER including tamoxifen, fulvestrant, and aromatase inhibitors (AI) (anastrozole, letrozole, and exemestane). Interestingly, endocrine therapy has been shown to act by restoring in part cell cycle control [49–51]. Although endocrine therapy is very efficacious, resistance commonly develops, representing a major clinical problem. The biology of resistance to endocrine therapy is complex and has not been completely elucidated, being underlain by deregulation of several different pathways [52]. Cyclin D1 overexpression and amplification in human breast tumors have been associated with resistance to tamoxifen [53, 54]. Interestingly, recent data has shown that breast cancer cells that have developed resistance to estrogen deprivation rely on an ER/CDK4/E2F axis for survival and that an E2F activation gene signature correlated with a lesser response to AI in breast cancer patients [55], therefore suggesting CDK4/6 as a possible target for reversing the resistant phenotype. In vitro and in vivo models demonstrated that breast cancers harboring functional inactivation of Rb overcome hormone deprivation therapy [56], and endocrine-resistant breast tumors maintain cyclin D1 expression [57] and Rb phosphorylation despite effective ER blockade [49]. Fulvestrant resistance has also been associated with CDK6 overexpression [58]. Finally, the transcription factor FOXM1, a well-known transcriptional target and regulator of ER [59] with a critical role in breast cancer endocrine resistance [60], has been shown to be modulated by CDK4/6 [61, 62].

Both preclinical and preliminary clinical studies suggest that CDK4/6 inhibitors are highly effective in HR-positive breast tumors and may have a role in endocrine-resistant breast cancers. In vitro studies demonstrated that ER-positive cell lines are the most sensitive to inhibition by palbociclib, with combination of palbociclib and tamoxifen demonstrating a synergistic effect [63]. Palbociclib was also shown to improve efficacy of fulvestrant and letrozole in ER-positive breast cancer models [64]. Additionally, palbociclib has shown activity in endocrine-resistant cell lines [49] and induced durable cell cycle arrest and partially restored tamoxifen sensitivity in MCF cells resistant to tamoxifen [63].

69.4.2 Clinical Data

Available clinical data confirm the activity of CDK4/6 inhibitors in patients with HR-positive breast cancer. Although palbociclib showed modest activity (clinical benefit rate of 21%) when used as a single agent in a phase II trial (NCT01037790) in heavily pretreated advanced breast cancer patients, partial responses and stable disease were observed in patients with ER-positive tumors [65]. Additional clinical data for palbociclib in combination with endocrine therapy in the metastatic setting come from the seminal randomized phase II PALOMA-1/TRIO-18 trial that was recently published. This study evaluated the combination of palbociclib plus letrozole as first-line treatment for postmenopausal patients with ER-positive/HER2-negative metastatic breast cancer as compared to single-agent letrozole. The study was composed of two parts: Part 1 enrolled patients with ER+/HER2-negative disease with no further biomarker assessment, while Part 2 enrolled patients with tumors harboring Cyclin D1 gene amplification and/or loss of p16. The primary endpoint was investigator-assessed PFS. A total of 165 patients were randomized in this study (66 patients in Part 1 and 99 patients in Part 2). Baseline characteristics were well balanced between treatment arms. The majority of the patients enrolled presented with de novo metastatic disease (52% in the palbociclib + letrozole arm and 46% in the letrozole arm) or recurred more than 12 months from the end of adjuvant treatment (30 and 37% in the combination arm and the letrozole arm, respectively). Roughly half of the patients in both arms had not received any systemic adjuvant/neoadjuvant treatment prior to study entry, while less than a third of patients had received prior endocrine therapy, of which almost half had received an aromatase inhibitor. Therefore, the patient population in this

trial represents mostly a population with predictably high benefit from first-line endocrine therapy in the metastatic setting. However, 44% of the patients in the combination arm and 53% in the letrozole arm presented with visceral disease, therefore representing a population enriched with patients with a poorer outcome. The final analysis of primary endpoint showed a statistically significant improvement in PFS for the combination arm (20.2 months) compared to the letrozole arm (10.2 months) with hazard ratio (HR) = 0.488 (95% CI: 0.319, 0.748, 1-sided $p = 0.0004$) [66]. Significant improvements in PFS were also seen in both Part 1 and Part 2 when analyzed separately (HR = 0.299 [95% CI: 0.156, 0.572]; 1-sided $p = 0.0001$ for Part 1 and HR = 0.508 [95% CI: 0.303, 0.853]; 1-sided $p = 0.0046$ for Part 2). The OS analysis with only 61 events was still immature and demonstrated a trend favoring the palbociclib plus letrozole combination vs. letrozole (37.5 months with 30 events vs. 33.3 months with 31 events, respectively; HR = 0.813; $p = 0.2105$).

The toxicity profile of palbociclib appears very favorable. In the abovementioned single-agent study, side effects were mainly hematological, in line with data from the phase I trials. Grade 3/4 toxicities were limited to transient neutropenia (50%) and thrombocytopenia (21%), and all other toxicities were grade 1/2. Treatment was interrupted in 25% and dose reduced in 46% of patients for cytopenia [65]. Similarly, in the PALOMA-1 study, the most common adverse events in the combination arm were neutropenia, leukopenia, fatigue, and anemia [66]. In particular, grade 3–4 neutropenia was reported in 54% of the patients in the combination arm and versus 1% of the patients in the letrozole arm. Of note, no cases of febrile neutropenia or neutropenia-related infections were reported, and discontinuation rates due to adverse events were 13% in the combination arm versus 2% in the letrozole arm. Comparable safety data deriving from the US Expanded Access Program for palbociclib that enrolled 242 patients were recently communicated in abstract form [67]. The results of the PALOMA-1 trial led to the approval of palbociclib by the Food and Drug Administration for the treatment of patients with HR-positive and HER2-negative advanced breast cancer in combination with letrozole. An ongoing phase III trial reproducing the same design of PALOMA-1 (PALOMA-2) is ongoing.

In the setting of endocrine-pretreated metastatic breast cancer, results from the phase III PALOMA-3 trial have recently been published [68]. This study involved patients with ER-positive/HER2-negative metastatic breast cancer that had relapsed or progressed during prior endocrine therapy. Patients were randomized in a 2:1 ratio favoring the combination arm, to receive palbociclib and fulvestrant or placebo and fulvestrant. Premenopausal or perimenopausal women were eligible and also received goserelin. Patients who had received one line of prior chemotherapy for the treatment of metastatic disease were eligible for this trial. A total of 521 patients were randomized in this study, 347 in the palbociclib and fulvestrant arm and 174 in the palbociclib and placebo arm. The majority of the patients were postmenopausal (79.3% in both arms) and had received one or more prior lines of therapy for metastatic disease (patients who had received no prior treatment for metastatic disease represented 24.2% and 25.9% of the trial population in the palbociclib + fulvestrant and the placebo + fulvestrant arms, respectively). The study met its primary endpoint of progression-free survival. Median progression-free survival was 9.2 months with palbociclib and fulvestrant and 3.8 months with placebo and fulvestrant (HR) = 0.42 (95% CI, 0.32–0.56) and $p < 0.001$ [68]. Toxicities were mostly in line with previous data. The rate of discontinuation due to adverse events was 2.6% with palbociclib and 1.7% with placebo. To date, overall survival data are still immature and require longer follow-up [68].

Additional data with other CDK4/6 inhibitors in the context of breast cancer come from a phase I clinical trial in which abemaciclib was given as a single agent to 47 patients with metastatic breast cancer who had received a median of seven prior systemic regimens (NCT01394016). Among 36 patients with HR-positive tumors, nine confirmed partial responses were observed, for an overall response rate of 25%, a disease control rate (complete response + partial response + stable disease) of 81%, and a median PFS of 9.1 months [34]. Based on these encouraging results, abemaciclib has been granted FDA breakthrough therapy designation for patients with refractory HR+ metastatic breast cancer. Another phase Ib trial investigating the safety and tolerability of abemaciclib in combination with endocrine or HER2 target therapies for pre- or postmenopausal patients with metastatic breast cancer was recently communicated in abstract form (NCT02057133). In this study, patients with HR+ and HER2-negative disease received abemaciclib in combination with either letrozole ($n = 20$), anastrozole ($n = 16$), tamoxifen ($n = 16$), exemestane ($n = 15$), or exemestane plus everolimus ($n = 17$). HR+/HER2-negative patients included in this study had not received any prior systemic chemotherapy for metastatic disease but had received a median number of prior systemic treatments that varied among the different cohorts between 2 and 4. This study did not show any evidence for pharmacokinetic drug-drug interactions between abemaciclib, tamoxifen, exemestane, and trastuzumab and therefore showed the potential for abemaciclib to be safely combined with other targeted and endocrine treatments. The toxicity profile of the combinations confirmed what had been seen with abemaciclib monotherapy, with diarrhea, nausea, vomiting, neutropenia, and fatigue being the most frequently reported adverse events. Activity was seen in all cohorts, with partial responses ranging from

10 to 27% with a disease control rate (complete response + partial response + stable disease) ranging from 54 to 88% and a PFS at 6 months ranging from 86.7 to 73.3% [69].

Ribociclib is also in advanced stage of clinical development. However, clinical data in patients with breast cancer have not been fully published yet. Available data come from the phase Ib part of a phase Ib/II trial of ribociclib in association with either letrozole, the alpha-isoform selective PI3K inhibitor alpelisib (BYL719), or both and have been communicated in abstract form (NCT01872260). Regarding the combination of ribociclib and letrozole, ten patients were treated showing no signs of pharmacokinetic interaction, a fairly acceptable toxicity profile with the more common adverse event reported being neutropenia (all grades 90%, G3–G4 50%) and nausea (all grades 40%, G3–G4 0%), and preliminary signs of efficacy with 1 PR and 2 SD out of six evaluable patients [70].

Many studies are currently investigating CDK4/6 inhibitors in combination with endocrine therapy in patients with HR-positive breast cancer, which will give a definitive answer on the efficacy and safety of the use of CDK4/6 inhibitors in this patient population. We are currently running a phase II, open-label, multicenter, randomized clinical trial of palbociclib monotherapy versus palbociclib in combination with the endocrine therapy to which the patient has progressed in the previous line for ER-positive, HER2-negative postmenopausal advanced breast cancer patients [To Reverse ENDocrine resistance (TREnd) trial] (NCT02549430). This trial has been designed primarily to address the open question of whether palbociclib is able to restore endocrine sensitivity in patients previously treated with endocrine therapy.

Clinical data for the triple combination of ribociclib, letrozole, and the alpha-isoform selective PI3K inhibitor alpelisib (BYL719) have been recently communicated in abstract form (NCT01872260) [73]. Within this trial (NCT01872260), 36 postmenopausal women with HR+ HER2-negative advanced breast cancer received the triple combination. Fifteen patients discontinued treatment: seven (19%) due to disease progression and eight (22%) due to adverse events, the most frequent being nausea (all grade, 44%; G3/4, 6%), hyperglycemia (44%; 17%), neutropenia (42%; 22%), and fatigue (36%; 11%). The triple combination showed some preliminary clinical activity. Indeed of 27 evaluable patients, two (7%) had PR, four (15%) had unconfirmed PR, and six (22%) had SD [73].

Another ongoing phase Ib/II clinical trial (NCT01857193) is investigating the combination of the triple combination of ribociclib, everolimus, and exemestane in women with anastrozole- or letrozole-resistant HR+/HER2-negative advanced breast cancer. Preliminary results of this trial have been recently reported in abstract form for 70 patients treated with the triplet [74]. Triple combination permitted lower dosing of everolimus (mostly at 2.5 mg), resulting in better tolerability. Grade 3/4 treatment-related adverse events were neutropenia (45.7%), leukopenia (8.6%), and thrombocytopenia (5.7%), and two (2.9%) patients discontinued due to adverse events. The triplet showed encouraging signs of clinical activity. Among 55 patients evaluable for best overall response, there was (1.8%) complete response (CR), two (3.6%) confirmed and three (5.5%) unconfirmed PR and 26 (47.3%) SD. Of note, clinical activity was also reported in some patients with prior exposure to PI3K/AKT/mTOR or CDK4/6 inhibitors [74].

69.5 Combinations of CDK4/6 Inhibitors and Other Targeted Agents in Breast Cancer

Activating mutations of PIK3CA gene, encoding for the p110 alpha subunit of PI3Kinase, are a common event in HR+ breast cancer, affecting 45% and 29% of luminal A and luminal B breast cancer subtypes, respectively [40]. As a consequence, strategies to target this pathway in combination with hormonal therapies have been developed. Results from clinical trials with the mTOR inhibitor everolimus (BOLERO-2) [71] have been encouraging; however resistance to these drugs eventually develops.

A recent preclinical study revealed, through a combinatorial drug screen on multiple PIK3CA mutant breast cancer cells, that the combination of CDK4/6 inhibitors with PI3K inhibition reduces cell viability [72]. In addition, CDK4/6 inhibitors sensitized cells with acquired and intrinsic resistance to PI3K inhibitors both in vitro and in vivo [72].

69.6 CDK4/6 Inhibitors in HER2-Positive Breast Cancer

HER2-positive breast cancers represent 10–15% of all breast cancers. Women with HER2-positive tumors are currently treated with anti-HER2 therapies including trastuzumab, lapatinib, and pertuzumab, often in combination with chemotherapeutic agents and trastuzumab emtansine (TDM-1). Mouse models demonstrated that cyclin D1-CDK4 complex is critical for (HER2) *neu*-induced tumorigenesis [18, 19, 75]. In addition, analysis of HER2-enriched human tumors showed that cyclin D1 amplification and CDK4 gains are frequent events in this breast cancer subtype [40], supporting the central role of cyclin D1-CDK4-Rb axes in this context.

Preclinical data suggest that HER2-positive tumors might benefit from CDK4/6 inhibition. Indeed, the preclinical work of Finn et al. revealed that HER2-positive breast cancer cells show sensitivity to palbociclib treatment in vitro [63, 76]. Palbociclib activity in HER2-positive tumors has been then

confirmed in primary breast tumor explants and in vivo xenograft models [77]. In addition palbociclib has been shown to act synergistically with trastuzumab in three HER2-amplified breast cancer cell lines [77] and to have highly distinct mechanisms of action compared to TDM-1, which could yield cooperative effects [77]. Interestingly, CDK4/6 inhibition was also effective at blocking proliferation in models of acquired resistance to lapatinib [77]. In a mouse model of HER2-positive breast cancer (MMTV-c-neu), treatment with palbociclib as a single agent caused a decrease in tumor proliferation, a marked reduction in tumor volume (with several tumors showing complete regression), and an increase in median survival [37]. In HER2-amplified cell lines, the combinations of palbociclib and trastuzumab or T-DM1 proved to be synergistic [63, 76]. However, coadministration of palbociclib with carboplatin or doxorubicin in the absence of anti-HER2 therapy showed an antagonistic effect [78].

To date, despite the encouraging preclinical data, clinical data regarding the efficacy of CDK4/6 in patients with HER2-positive breast cancer are lacking. Given the biological rationale and the clinical need for new treatment strategies for patients with HER2-positive breast cancer, this is certainly an open research question.

69.7 CDK4/6 Inhibitors in Triple-Negative Breast Cancer (TNBC)

Approximately 10–15% of all breast cancers do not express HRs or HER2, thus belonging to the TNBC category. TNBC is an aggressive disease characterized by high proliferation rate; therefore targeting the cell cycle might be a potential therapeutic strategy in these patients. Among other molecular characteristics, TNBCs often exhibit loss/mutation of RB and high expression of p16 which are characteristics usually associated with resistance to CDK4/6 inhibition [40]. To date it is still unclear whether patients with TNBC can derive any benefit from CDK4/6 inhibitors; however the majority of preclinical findings suggest that TNBCs do not respond to CDK4/6 inhibition.

Analyzing drug sensitivity in a panel of breast cancer cell lines, it was demonstrated that most of basal-like cell lines (which recapitulate TNBC biology) are resistant to palbociclib [63]. Also, a recent study investigating the regulatory role of Cyclin D1 and CDK4/6 inhibition on migration and stemlike cell activity showed that results vary depending on ER status of tumor cells [79]. Inhibition of cyclin D1 or CDK4/6 led to decreased migration and stemlike activity in ER-positive breast cancer cells but showed opposite effects on ER-negative cell lines. Palbociclib treatment reduced mammosphere formation in ER-positive breast cancer cells, while in ER-negative cells, mammosphere formation was instead increased [79]. Re-expression of ER in two ER-negative cell lines was sufficient to overcome these effects [79]. Additionally, in a basal-like Rb-deficient breast cancer mouse model (C3-Tag model), palbociclib treatment alone did not affect tumor proliferation or growth [78]. On the other hand, it must be mentioned that some of the basal-like cells analyzed by Finn et al. [63] did show moderate sensitivity to palbociclib (HCC-38 and BT-20 with IC50 values of 64 and 177 nM, respectively) and palbociclib was shown to strongly inhibit cell proliferation of some TNBC cell lines [80]. Additionally, in an ex vivo analysis of 13 human tumors, response to palbociclib was demonstrated not to be dependent on ER or HER2 status [81]. TNBC is a heterogeneous disease; therefore response to CDK4/6 inhibitors might depend on intrinsic biological characteristics. Indeed, it has been demonstrated that palbociclib can induce G1 cell cycle arrest in Rb-proficient TNBC cell lines [82, 83] yet is completely ineffective at suppressing proliferation in Rb-deficient TNBC cells [83].

Patients with TNBC are often treated with chemotherapeutic agents including anthracyclines, taxanes, or platinum salts. The efficacy of CDK4/6 inhibition in combination with such agents has therefore recently been tested. Again the effects were shown to be dependent on Rb status [82, 83]. In Rb-proficient TNBC cells, palbociclib antagonized the cytotoxic activity of anthracyclines and taxanes by preventing the induction of DNA damage and consequent cell death and preserved cell viability of doxorubicin-treated cells [82, 83]. In Rb-deficient TNBC cells and mouse models, in which palbociclib alone had no effect, the combination did not alter the therapeutic response to chemotherapeutic agents [78, 82, 83]. Overall, these data do not support the association of CDK4/6 inhibitors with chemotherapy in breast cancer. However, it must be mentioned that a combination of abemaciclib and the chemotherapeutic agent gemcitabine in a xenograft model of lung cancer (calu-6 cells) resulted in additive antitumor activity in comparison to single agents, even though no cell cycle arrest was detected [31].

To date, the only clinical data on the role of CDK4/6 inhibitors in TNBC derive from the abovementioned phase II trial (NCT01037790) in which palbociclib was administered as single agent in patients with refractory tumors. Seven of the eight patients enrolled with TNBC progressed, and only one patient had disease stabilization. Of note all patients enrolled in the trial had Rb-positive tumors [65].

69.8 Potential Biomarkers of Response to CDK4/6 Inhibitors

It is evident that CDK4/6 inhibitors represent a new paradigm for the treatment of metastatic breast cancer patients with HR-positive breast cancer, and available clinical data

suggest that virtually every patient with HR-positive breast cancer should be treated with a combination of endocrine therapy and a CDK inhibitor in the early metastatic setting. However, it must be taken into account that a relevant proportion (±50%) of ER-positive patients undergoing first-line single-agent hormonal treatment can achieve disease responses lasting more than 12 months, with minimal side effects [84, 85]. In this group of patients with high sensitivity to endocrine therapy, any additional benefit given by combinations with a CDK4/6 inhibitor must be carefully weighed, taking into account additional toxicity and costs.

In this context, understanding which subgroup of patients is more likely to benefit from the combination of endocrine therapy and CDK4/6 inhibitors is therefore of critical importance for the future clinical development of these agents.

Recent studies suggest that alterations in the CyclinD/CDK4/Rb pathway might have a predictive role for response to CDK4/6 inhibitors. However, to date, no single biomarker has been developed with any positive or negative predictive value for response to CDK4/6 inhibitors. Palbociclib has been associated with favorable PFS in CDK4-amplified well-differentiated or dedifferentiated liposarcoma [86] and in cyclin D1-overexpressing mantle cell lymphoma [87]. However, CDK4 amplification is not a frequent event in breast cancer. In breast cancer, genetic loss of RB and high expression of p16 protein, which is frequently associated with RB loss, have been linked to resistance to palbociclib [81], while high levels of Rb and cyclin D1 and low levels of p16 have been associated with sensitivity to this compound [63]. Rb expression was an inclusion criterion for the above-mentioned (NCT01037790) trial investigating palbociclib in patients with advanced solid disease [65]. However, despite the presence of functional Rb in tumors, the clinical benefit rate for patients with breast cancer was very modest [65]. Additionally, in the Part 2 of the PALOMA-1 trial, where all included patients were screened for cyclin D1 amplification and/or loss of p16, there was no indication of increased activity of palbociclib compared to Part 1 of the study where no molecular screening was requested [66]. More complex alterations of the pathway are therefore likely to be implicated in resistance to CDK4/6 inhibitors. Indeed, a wider analysis of the Rb/E2F pathway suggested that resistance to palbociclib might be mediated by other components of the pathway, such as E2F2, p107, CDK2, p21, and p27 [80]. Gene expression signatures that analyze the Rb pathway have been developed by different groups [88, 89] and shown to be prognostic in patients with breast cancer and potentially predictive of response to chemotherapy and endocrine therapy [88, 89]. These signatures of Rb functional state show a positive correlation with RB1 mutational status. It is therefore interesting to speculate that such signatures could be also predictive of resistance to CDK4/6 inhibitors.

Conclusion

CDK4/6 inhibitors look very promising for the treatment of patients with hormone receptor-positive HER2-negative breast cancer. Although well tolerated, these drugs are not devoid of side effects. Clearly, biomarker-driven clinical trials are urgently needed to identify subpopulations of breast cancer patients that will derive the greatest benefit from CDK4/6 inhibitors.

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Targeting FGFR Pathway in Breast Cancer

70

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70.1 Introduction

The fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) signaling pathway plays a critical role in several cancer types, including breast cancer.

Deregulated FGF signaling is involved in cancer progression in tumors driven by FGF/FGFR oncogenic mutations or amplifications. The FGF/FGFR alterations that occur in cancer may result either in constitutive ligand-independent FGFR activation or in abnormal ligand-dependent signaling.

FGFR pathway activation is generally involved in cancer progression through oncogenesis, neoangiogenesis, and drug resistance. Therefore, there is a strong biologic rationale to support the development of anti-FGF/FGFR agents in breast cancer.

The main activating FGFR genomic alterations found in breast cancer concern FGFR1 and FGFR2.

The amplification of the chromosomal region 8p11–12, which includes the gene encoding

FGFR1, has been identified in 8–10% of breast cancers, mostly in estrogen receptor (ER)-positive tumors. Additionally, this molecular alteration is related to higher FGFR1 mRNA levels [1, 2] and poorer prognosis [3, 4]. Breast cancer cells with FGFR1 amplification are extremely sensitive to FGFR1 tyrosine kinase inhibitors [5]. While FGFR2 amplification has been identified in 4% of triple-negative breast cancers. It has been shown that PD173074 treatment induced apoptosis in FGFR2-amplified cell lines of triple-negative breast cancer, partially owing to inhibited PI3K/AKT signaling [6]. Components of the FGF pathway have been particularly identified in mesenchymal and mesenchymal-like subtypes. Also, cell models of these specific subtypes have been proven to be sensitive to PI3K inhibition. Other interesting information come from genome-wide association studies, which have identified FGFR2 as a breast cancer susceptibility gene; indeed, several single nucleotide polymorphisms (SNP) in FGFR2 are highly associated to breast cancer risk [7]. Having this said, it is easily understandable why there is so much interest in conducting effective trials with FRGF inhibitors in breast cancer.

Here, we present a critical overview of the last year literature focusing on the rationale and potential role of FGFR inhibitors in breast cancer.

70.2 Review

70.2.1 Predictive Factors of Response to FGFR Inhibitors

Identifying the patient population who might derive the greatest benefit from FGFR pathway-targeted therapy is paramount but still challenging and controversial. The first tricky point is the definition of FGFR pathway amplification, as different methods and different threshold copy number have been used across studies. A quantitative gene amplification measurement may not accurately reflect protein expression or activity. Activating mutations may not be relevant if the gene is not expressed. Furthermore, amplicons may not reflect the level of amplification of the component genes, and other potential oncogenes in the amplicon may represent confounding factors. So far it is still unknown which is the best method to test the FGF pathway amplification, if any. Therefore, ongoing clinical trials are using different approaches to detect FGFR alterations. This will ultimately result in potential biases when current data will be analyzed. One of the most demanding but necessary step in this field is to fine-tune the selection of patients more likely to benefit from FGFR-targeted therapies.

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Table 70.1 Trials with FGFR inhibitors in metastatic breast cancer

Drug	Phase	Study number	Status	Therapy
Lucitanib	1/2	NCT01283945	Active, not recruiting	Lucitanib [8]
Lucitanib	2	NCT02053636	Recruiting	Lucitanib
Dovitinib	1/2	NCT01484041	Active, not recruiting	Dovitinib + aromatase inhibitor
Dovitinib	2	NCT00958971	Completed	Dovitinib
Dovitinib	2	NCT01528345	Completed	Dovitinib + fulvestrant
Dovitinib	2	NCT01262027	Recruiting	Dovitinib
AZD4547	1/2	NCT01202591	Completed	AZD4547 + fulvestrant vs. fulvestrant alone
AZD4547	1/2	NCT01791985	Recruiting	AZD4547 + (anastrozole or letrozole) versus exemestane
AZD4547	2	NCT01795768	Recruiting	AZD4547
BGJ398	1	NCT01004224	Recruiting	BGJ398
BGJ398	1	NCT01928459	Recruiting	BGJ398 + BYL719
JNJ-42756493	1	NCT01703481	Recruiting	JNJ-42756493
JNJ-42756493	1	NCT01962532	Recruiting	JNJ-42756493
Brivanib	1	NCT00798252	Completed	Brivanib + chemotherapy
Orantinib	2	Not available	Completed	Orantinib [9]
Orantinib	2	Not available	Completed	Orantinib + docetaxel [10]
Nintedanib	2	NCT01658462	Recruiting	Docetaxel +/−nintedanib

70.2.2 FGFR Inhibitors Under Investigation in Breast Cancer

A number of FGFR inhibitors are investigated in clinical trials (Table 70.1).

Dovitinib (TKI258) is a potent tyrosine kinase inhibitor that targets FGFR, vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR), and other kinases. It has been mainly investigated in patients with metastatic ER+/HER2− breast cancer. After the first evidence of activity [11], trials of dovitinib in combination with endocrine therapies have been designed (NCT01528345, NCT01262027, NCT01484041).

AZD4547 inhibits FGFR1-3 and VEGFR2 [12]. Similarly to dovitinib, this agent is being tested in patients with ER+ breast cancer either alone or in combination with endocrine therapies (NCT01795768, NCT01202591, NCT01791985).

Pan-FGFR inhibitors – such as BGJ398 and JNJ-42756493 – are under clinical investigation in patients with tumors harboring FGFR1/FGFR2 amplifications or FGFR3 mutations (NCT01004224, NCT01962532). These molecules seem to induce reductions in tumor volume, both when used as monotherapy and when combined with the α-selective PI3K inhibitor BYL719 in patients with tumors harboring PIK3CA mutations and FGFR1-3 alterations (NCT01928459, NCT01703481).

A program for the development of lucitanib, – a potent, oral inhibitor of FGFR 1 and 2, VEGFR 1, 2 and 3, and PGFRα/β – is ongoing, but we will discuss this drug in the next paragraph.

Other FGFR inhibitors tested in breast cancer include – but are not limited to – orantinib (TSU-68), which inhibits VEGFR2, PDGFR, and FGFR [9], brivanib alaninate (NCT00798252), and nintedanib (BIBF 1120), which inhibits FGFR1-3, VEGFR1-3, PDGFRα, PDGFRβ, and FLT3 [13].

70.2.3 2014 News and Views

The role of FGFR inhibition on tumor growth and metastasis in breast cancer is well known [14]. Previous studies demonstrated that the FGFR inhibitor PD173074 decreased the viability of numerous human breast cancer cells and 4T1 murine mammary tumor cells [15]. Hence, in a 2014 preclinical paper, it has been shown that PD173074 induces 4T1 cell apoptosis in a concentration-dependent manner [16]. Apoptosis induced by PD173074 was linked to the inhibition of Mcl-1 and survivin. Also, PD173074 considerably increased the Bax/Bcl-2 ratio. In vitro, PD173074 blocked 4T1 cell migration and invasion too. Additionally, in 4T1 tumor-bearing mice, PD173074 significantly inhibited tumor growth, reduced microvessel density and proliferation index, and induced tumor apoptosis. FGFR inhibition induced by PD173074 reduced myeloid-derived suppressor cells in the blood, spleens, and tumors, with increased CD4(+) and CD8(+) T cells infiltration in the spleens and tumors. Furthermore, metastasization to the lung was significantly inhibited by PD173074. Taken together, these findings support the importance of capitalize upon FGFR inhibition as a therapeutic strategy for breast cancer, as it delays tumor

progression, impedes lung metastasization, and cracks immunosuppression.

Moving forward, a clinical phase I/IIa trial evaluated the safety, efficacy, pharmacokinetics, and pharmacodynamics of lucitanib in advanced solid tumors, including breast cancer [8]. Seventy-six patients were included and treated with doses from 5 to 30 mg. The main dose-limiting toxicity was related to the VEGF inhibition. Namely, the most common adverse events were hypertension (91%), asthenia (42%), and proteinuria (57%). Clinical activity was observed at all doses with durable partial responses according to Response Evaluation Criteria In Solid Tumors (RECIST) criteria. In patients with breast cancer and FGF aberration, 50% achieved partial responses with a median progression-free survival of 40.4 weeks. Therefore, lucitanib is a promising drug for FGF+ breast cancer.

Although the majority of data published so far concern the inhibition of FGFR1 and 2, a paper published last year provided insights on the structural analysis of the FGFR4 [17]. Normally, for FGFRs and specifically for FGFR1, there is the phosphorylation of the internal tyrosine 653 first and then the phosphorylation of the external tyrosine 654 [18, 19]. Authors of this preclinical work identified an autoinhibition "dual switch" mechanism for FGFR4 kinase domain. This means that the kinase can be less autoinhibited and achieve a partially active conformation when both the tyrosines of the activation segment are sequentially phosphorylated and stepwise relocated [17]. The identification of these mechanisms shows that FGFR4 behaves differently from other FGFRs. Authors of this paper identified four structures of the kinase domain of FGFR4, in its apo-form and combined with different small-molecule inhibitors [17]. The two apo-FGFR4 kinase domain structures present an activated segment comparable to an autoinhibitory segment observed of the hepatocyte growth factor receptor kinase but different from the other FGFR kinases [17]. The observation of molecular interactions between FGFR4 and different types of kinase inhibitors such as the type I inhibitor dovitinib and the type II inhibitor ponatinib might potentially lead to the design and development of FGFR4 inhibitors for breast cancer [17].

Such an observation may be even more interesting if we consider that resistance to first-generation FGFR kinase inhibitors may occur. Resistance is usually induced by selection for mutant kinases unreceptive to the drug action or by upregulation of compensatory signaling pathways [20–22]. Specifically, resistance to FGFR inhibitors can occur through mutations in the FGFR gatekeeper residue [23–25].

In this context, it is worth mentioning another paper published in 2014 on the development of two selective, next-generation covalent FGFR inhibitors, the FGFR irreversible inhibitors 2 and 3 [26]. These two drugs inhibit the proliferation of cells dependent upon the gatekeeper mutants of FGFR1 or FGFR2, which cause resistance to first-generation FGFR inhibitors [26]. Because of the cocrystal structure of FGFR4 with FGFR irreversible inhibitor 2, a "Asp-Phe-Gly (DFG)-out" covalent binding mode has been displayed. FGFR irreversible inhibitor 3 – due to the conformational flexibility of the reactive acrylamide substituent – covalently inhibits both the EGF receptor (EGFR) and FGFR by targeting two different cysteine residues. Moreover, crystal structures of FGFR irreversible inhibitor 3 bound with FGFR4 V550L and EGFR L858R explain the dual FGFR and EGFR targeting by FGFR irreversible inhibitor 3. Therefore, this study – besides showing the potential of a kinase inhibitor able to covalently target different cysteines within the ATP-binding pocket – highlights the importance of covalent FGFR inhibitors in overcoming resistance to this class of drugs.

Conclusion

The FGFR pathway – besides being involved in several physiologic processes – has a paramount role in many tumor types, including breast cancer. In the era of personalized medicine, it is very important to understand the mechanisms through which the FGFR pathway drives the disease. Also, it is even more important to understand how to capitalize upon the FRGF inhibition in the treatment of breast cancer. Two of the main goals of the next clinical trials with these compounds should be the better identification of patients with FGFR pathway-amplified tumors who are more likely to respond to FGFR inhibitors and the development of new FGFR inhibitors able to overcome resistance to first-generation FGFR inhibitors.

Key Points

1. Aberrant FGFR signaling is known to be involved in the pathogenesis of breast cancer.
2. FGFR targeting has improved over the last years due to the development of novel agents inhibiting FGF or FGFR, especially in breast cancer with FGF aberrations.
3. Right now, there is increasing interest in developing new FGFR inhibitors able to overcome resistance to first-generation FGFR inhibitors.
4. The selection of patients who might derive the greatest benefit from FGFR inhibitors is paramount.

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Integrating Next-Generation Sequencing Data in Trial Design

71

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71.1 Introduction

The understanding of the central role of the *ERBB2/HER2* gene, amplified in approximately 15–20% of breast cancer, has altered the natural history of this aggressive subtype of breast cancer with the development of therapies directed against the HER2 receptor, such as trastuzumab, lapatinib, pertuzumab, and trastuzumab-DM1. There is an exciting array of experimental breast cancer therapies directed against novel targets that are currently in clinical development. These investigational agents are likely to be effective for small subsets of breast cancers with specific "driver mutations." The ability to perform comprehensive molecular profiling of individual tumors has rapidly expanded over the last few years, as the cost DNA sequencing technologies that allow for targeted multiplex "hotspot" mutation testing or deeper targeted exome and whole genome DNA sequencing has become cheaper than traditional Sanger-based DNA sequencing methods. New DNA sequencing technologies require relatively limited quantities of fresh or archived paraffin-embedded or snap frozen tumor tissue and provide rapid turnaround of sequencing results within a few weeks or less. These technological advances allow for the prospect of point-of-care molecular profiling that can be used to guide the development of personalized breast cancer medicine therapy. For an international collective of academic breast cancer researchers, this provides an unprecedented opportunity to identify patients with rare "driver" molecular alternations that are candidates for proof-of-concept clinical trials with matched targeted therapy. The aim of this report on molecular profiling is to review the known recurrent molecular alterations in breast cancer that are potentially amenable to investigational targeted therapy, to provide an overview of the existing technological platforms for molecular profiling and ongoing or planned institutional/national screening initiatives, and to outline a vision for molecular screening that may be integrated into the future activities of breast cancer research.

71.2 Background and Rationale

Personalized medicine and new drug development. The "oncogene revolution" has led to an explosion of molecularly targeted therapeutics in preclinical and clinical development over the last decade [1]. It is estimated that there are more than 800 targeted anticancer therapies currently in various stages of clinical development. Disappointingly, historical data indicate that only 5% of these investigational therapies will ultimately progress to registration for widespread use. These high attrition rates have multiple causes, including lack of efficacy and excessive toxicity [2]. In particular, when patients are selected for phase III trials based on histopathology alone, a targeted drug with a 5–10% single-agent response rate runs a high risk of failure [3]. Recent efforts to systematically sequence cancer genomes have revealed that individual tumors frequently harbor multiple "driver" somatic mutations that confer growth advantage and positive selection [4].

The increasing identification of specific somatic mutations and other genetic aberrations that drive cancers leaves us on the threshold of a new era of "personalized cancer medicine," in which specific biomarkers will be used to direct targeted agents only to those patients deemed most likely to respond. The potential medical, scientific, and economic benefits of such a personalized approach to cancer therapy are immense and self-evident. Yet despite some important advances, only a limited number of approved targeted agents have had their approvals predicated on specific biomarkers of sensitivity or resistance. The premises behind personalized cancer medicine include the following: (i) genetic aberrations exist in human malignancies; (ii) a subset of these aberrations, often present

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across multiple cancer types, have functional relevance as "drivers" for oncogenesis and tumor progression; (iii) such genetic aberrations are potentially "druggable" targets; and (iv) there are tolerable medicinal compounds that can effectively modulate such targets [5]. A key requirement of this new, personalized approach to anticancer therapy is that specific patients must be matched to a particular drug or combination of drugs. Molecular profiling of tumors to identify somatic mutations and/or other genetic aberrations are examples of enrichment strategies to assist in matching patients to drugs or treatments that have gained increasing interest in the oncology community [6]. The true merits of such personalized medicine strategies remain to be established. However, proof-of-concept clinical trials that establish the value of matching targeted treatments to rare molecular alterations in breast cancer and other malignancies are beyond the scope of any single pharmaceutical sponsor, cancer treatment facility, or national cancer agency and will ultimately require international collaboration. Recent examples demonstrate that sequential testing of infrequent genomic alterations to identify candidates for clinical trials with matched targeted is inefficient, expensive, and wasteful of scarce archived tumor tissue resources. Comprehensive molecular screening programs, which provide simultaneous testing of multiple biomarkers early in the course of a patient's natural history of disease, are most likely to advance personalized cancer medicine.

Genomic alterations in breast cancer. Somatic mutations are responsible for approximately 90% of breast cancers. Although data from comprehensive, large-scale breast cancer DNA sequencing projects are still awaited [7], key features of the genomic breast cancer landscape have begun to emerge. First, although multiple regions of copy gain are observed, none occurs as frequently as 17q12 which harbors *ERBB2/HER2*; second, there are high-frequency somatic point mutations in three "gene mountains" [5]—*TP53* (44%), *PIK3CA* (26%), and *CDH1* (19%)—but low-frequency recurrent point mutations (<5%) are also seen in genes that are validated drug targets in other types of cancer (i.e., *KRAS*, *BRAF*, and *EGFR*); third, genes with somatic point mutations are also frequently regions of copy number gain in independent tumor samples (i.e., *PIK3CA*, *ERBB2*), highlighting their importance as oncogenes; and fourth, point mutations are observed in multiple components of a signaling pathway at a higher rate than expected by chance alone (i.e., *PIK3CA*, *PTEN*, *AKT1*) indicating the relevance of the signaling pathway as a therapeutic target in mutated tumors. Additional data from large-scale sequencing projects, such as The Cancer Genome Atlas (TGCA) and the International Cancer Genome Consortium (ICGC), should provide additional insight with regard to the characteristic genome alterations that define the intrinsic molecular subtypes of breast cancer.

71.3 Molecular Screening Programs

Clinical application of targeted genomic sequencing. Recent advances in DNA sequencing technology allow for rapid testing of multiple hotspot mutations using limited quantities of tumor DNA isolated from archival paraffin-embedded tumor material at an affordable cost [8–10]. Studies by Thomas et al., MacConaill et al., and Dias-Santagata et al. examined between 250 and 1000 individual tumor specimens for 120–400 mutations in 13–33 known oncogenes and tumor suppressor genes. These studies found at least one mutation in 30–37% of tumor samples. Recently, Sequist et al. published their experience at Massachusetts General Hospital (MGH) with molecular screening of 552 non-small cell lung cancer patients using the multiplex PCR-based SNaPshot assays, which detect ~50 mutations and 14 genes, and FISH for ALK translocations [11]. They identified ≥1 mutation in 51% of patients who underwent successful profiling and directed 70 (22%) of 353 patients with advanced disease to a genotype-directed therapy. There are two reported studies that have investigated if therapy matched to molecular profile (MP) improves outcome. Von Hoff et al. conducted a study of matching treatments to MP in 86 patients across nine different centers in the United States [12]. Only 66 patients proceeded to MP, wherein 64 targets were examined using a combination of immunohistochemistry (IHC), FISH, and gene expression microarrays. Each aberration was matched to a predefined treatment. In 18 of 66 patients, they demonstrated progression-free survival (PFS) for matched treatment to be 1.3 times greater than PFS for the treatment patients received immediately prior. Tsimberidou et al. performed molecular analysis on 1283 patients, with success in 1144 (89%) [13]. They used polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), and immunohistochemistry (IHC) in examining 11 separate molecular aberrations. In their cohort, 40% of patients had at least one aberration. They matched each aberration to a targeted treatment when available and demonstrated that patients who received matched targeted therapy had better response rates and improved time to treatment failure.

Molecular screening platforms. The advantage of multiplex PCR-based platforms such as Sequenom OncoCarta or OncoMap and Applied Biosystems SNaPshot assay is that they provide excellent coverage of frequently mutated "druggable" oncogenes when mutations cluster in a limited number of DNA sequence regions, such as *KRAS* (nine bases account

for >99% of all mutations), *BRAF* (15–18 bases account for >90% of all mutations), and *PIK3CA* (12–15 bases account for >80% of all mutations). However, for clinically relevant tumor suppressor genes, such as *TP53*, *PTEN*, *BRCA1*, or *BRCA2*, where mutations are more widely distributed across a much larger DNA coding region, the ability to detect mutations is limited to a few selected hotspots. In addition, the published molecular screening panels using these platforms are only able to detect known base-pair substitutions and limited deletions or insertions (indels) and gene amplification. They do not include translocations, larger indels, or novel base-pair substitutions. The Sequenom MassARRAY Analyzer has developed methods to evaluate copy number variation (CNV); however, this has not been validated for point-of-care molecular profiling using human tumor samples.

Next-generation sequencing. Sequenom, SNaPshot, and other PCR-based multiplex assays are constrained by bandwidth and throughput. Next-generation sequencing (NGS) refers to technological platforms that allow for massive parallel sequencing of millions of DNA templates. "Second"-generation deep sequencing refers to clonal amplification of DNA templates on a solid support matrix followed by cyclical sequencing with short reads. These instruments are currently used to sequence entire genomes, exomes, transcriptomes, and methylomes that often require weeks for sample template preparation, sequence generation, and data analyses. As a result, their use is largely confined to large genome centers. Since "second-generation" DNA sequencing instruments are not employed in diagnostic settings, additional validation of potential candidate mutations is required using clinical-grade sequencing assays in certified diagnostic laboratories. The advent of "third"-generation sequencers such as Pacific Biosciences PacBio RS and Life Technologies' Ion Torrent Personal Genome Machine (PGM) provides increased speed of sequencing due to their use of sensors that detect nucleotides as they are added to DNA molecules in synthesis, although parallelization and machine throughput currently are much lower than with second-generation technologies. In addition to the Ion Torrent PGM, other so-called "bench sequencing" machines have recently been released by Illumina (MiSeq) and Roche/454 (GS Junior), which are moderate throughput platforms with fast run times, long DNA reads, and automated library preparation that are well suited to clinical applications. The appeal of these low-cost (≤125,000€ per instrument) "bench sequencing" platforms is that they offer the opportunity to comprehensively test a large targeted panel of relevant cancer genes (1000 or more) with 30–50× or greater coverage to identify rare (<5% prevalence) mutations and copy number alterations that are potentially relevant to clinical care with a rapid turnaround time to results of 1 week or less. One of the major obstacles to NGS for cancer diagnostics is the ability to assess DNA extracted from limited formalin-fixed paraffin-embedded (FFPE) material, such as archival tumor blocks or small core tumor biopsies. Preliminary experience suggests that NGS is feasible from FFPE core tumor biopsies, although the quality of DNA isolated from archival tumor material that is routinely stored for >5 years and the robustness of methods of sequence enrichments remain questionable.

Ongoing molecular screening programs. Recognizing that cancer genome sequencing is likely to be integrated in routine clinical decision-making in the near future, many leading cancer research institutions and national cancer agencies have recently launched or are soon to launch broad-scale molecular screening programs for solid tumors, including breast cancer [14]. Massachusetts General Hospital (MGH) has implemented a phased rollout of the SNaPshot testing (which now includes ~120 mutations in 16 oncogenes) using archival tumor tissue in four tumor types: lung, colon, breast, and glioblastoma multiforme (GBM). The Vanderbilt-Ingram Cancer Center (VICC) also initiated a similar program of SNaPshot screening of archival tumor tissue in non-small cell lung cancer and melanoma in 2010 including ~40 mutations in 6–8 genes. They integrated the molecular screening results into the patient's electronic medical record. Their "My Cancer Genome" (www.mycancergenome.org) website includes information about common activating mutations in "druggable" oncogenes and includes links to clinical trials with molecular selection based upon molecular profiling. In July 2011, they expanded their program to include PI3-kinase pathway-specific mutation panel for breast cancer. The Dana-Farber Cancer Institute (DFCI) in partnership with the Brigham and Women's Hospital has recently announced an ambitious USD43 million program (PROFILE) to perform mutation profiling using OncoMap (which includes ~470 mutations in 41 genes) in selected tumors, including colon, lung, breast, and some sarcomas and leukemias. Their project will include patients with early-stage and advanced disease, linking genomic information with clinical outcomes and response to matched targeted therapies. It has been estimated that the program will include up to 10,000 patients annually [14]. In Canada, the Ontario Institute for Cancer Research (OICR) and Princess Margaret Hospital (PMH) opened a pilot feasibility with biopsy of metastatic lesions involving patients with advanced solid tumors for profiling using the Sequenom OncoCarta (v1.0) and the third-generation NGS platform PacBio RS analyzer for the same 19 genes as are included on the OncoCarta v1.0 panel. The initial results for the first 30 patients accrued were presented at the 2011 AACR-NCI-EORTC Molecular Target and Cancer Therapeutics Meetings [15]. PMH will soon launch its own internal program entitled the Integrated

Molecular Profiling in Advanced Cancers Trial (IMPACT) to perform mutation profiling using a customized Sequenom panel that includes ~277 mutations in 25 genes for patients with advanced non-small cell lung cancer, colorectal cancer, ovarian cancer, and breast cancer and patients considered for phase I clinical trials. The IMPACT study will initially include 500 patients annually and will be expanded to include additional disease sites and NGS technology.

Investigators at the University of Michigan also recently published their pilot experience with real-time high-throughput whole-exome sequencing for two patients enrolled in the MI-ONCOSEQ protocol [16]. They successfully performed whole-exome sequencing of fresh tumor biopsies from two patients—with colorectal cancer and melanoma—on the Illumina HiSeq platform and reviewed the results at a sequencing tumor board within 4 weeks from the time of tumor biopsy. There are plans to perform deep whole-exome sequencing of approximately 100 patients with advanced solid tumors per year, with the aim of matching patients to investigational clinical trials with targeted therapies. In Europe, there are also molecular screening programs that are underway. At the Institut Gustave Roussy (IGR) in Paris, the ongoing Molecular Screening for Cancer Treatment Optimization (MOSCATO) clinical trial protocol will perform molecular profiling using array comparative genome hybridization (aCGH) and Sanger sequencing for selected mutation hotspots in 600 patients over 3 years who are candidates for phase I clinical trials. Similarly, the ZAFIR01 clinical trial protocol at IGR will perform aCGH and targeted Sanger sequencing (PIK3CA and AKT1) in 400 patients with advanced breast cancer who undergo tumor biopsies for molecular screening. Cancer Research UK has recently launched the "Stratified Medicine Program" across seven cancer research hospitals in the United Kingdom which will perform molecular profiling for ~20 alterations in eight genes using archival tumor material from 9000 patients with advanced melanoma and breast, prostate, ovarian, colorectal, and non-small cell lung cancer over 2 years. The details of the platform that will be used for molecular profiling have not been publicly disclosed. In the Netherlands, hospitals from Amsterdam, Rotterdam, and Utrecht have launched a molecular screening to perform next-generation sequencing of fresh tumor biopsies from patients who are candidates for phase I clinical trials. Approximately 1200 patients will be enrolled over the next 3 years, with plans to profile approximately 2000 genes per patient using targeted sequencing on the Illumina HiSeq platform. The Breast International Group is also running a molecular screening program in metastatic breast cancer named AURORA project. AURORA has two broad purposes: (1) The first is to analyze breast cancer samples using techniques including but not limited to targeted DNA sequencing and RNA sequencing, in order to better understand the genetic aberrations related to breast cancer. This part of AURORA could help us understand breast cancer disease evolution (this will be done in all patients) and determine why some patients respond well to a certain treatment while others don't (this will only be done in a minority of patients). This may not provide you with any benefit directly, but your participation is likely to help us find answers to questions which could help to improve the treatment and/or quality of life of future breast cancer patients. (2) The second is to identify patients potentially eligible to participate in approved studies testing new therapeutic strategies based on known breast cancer-related molecular aberrations found in the breast cancer samples. Such identification is done when the aberrations of your primary and/or metastatic tumor DNA found by targeted sequencing match an ongoing therapeutic clinical trial testing a drug against the aberration. These trials might not be available at the time being, but your treating physician shall inform you in case they become available. If you are found to be eligible for an ongoing trial, your treating physician will give you more information and an additional informed consent form to sign, specific to that particular trial. Note that enrollment in one of these candidate trials is completely up to you. Please note that aberrations for which therapeutic clinical trials are available may be found only in a minority of patients. To start with, this research project will involve 1300 patients from hospitals mainly located in Europe.

71.3.1 Future Perspectives

It is likely that future clinical trials in breast cancer with targeted therapies will be conducted in molecularly defined subpopulations of disease. Advances in high-throughput DNA sequencing technology allow for screening a large number of genes simultaneously at a relatively low cost to molecularly characterize individual tumors for triage of clinical trials with targeted therapies. These molecular screening programs are rapidly being developed by large cancer research hospitals and national cancer societies in North America and Europe. It is very unlikely that a single pharmaceutical sponsor will be able to support the large-scale molecular screening programs to identify relatively rare subpopulations ($\leq$5%) of breast cancer that are amenable to clinical trials with matched targeted therapies. The existing model of sequential prescreening for individual clinical trials—with separate informed consent forms, processes of tumor material retrieval and shipping, and methods of laboratory testing and reporting—is expensive, inefficient, and not well suited to the current era of molecularly targeted drug development. We need to find new paths to access innovations to clinical research and daily practice. To ensure that continued innovation meets the needs of patients, the therapeutic alliance between patients and academic-led research should be extended to include relevant

pharmaceutical companies and drug regulators with a unique effort to bring innovation into clinical practice. We need to bring together major players from the world of breast cancer research to map out a coordinated strategy on an international scale, to address the disease fragmentation, to share financial resources, and to integrate scientific data. The final goal will be to improve access to an affordable, best standard of care for all patients in each country.

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Part XI

Investigational and Miscellaneous Approaches

Lifestyle and Breast Cancer

72

Rowan T. Chlebowski

The potential for lifestyle factors to influence breast cancer incidence, breast cancer recurrence risk, and breast cancer overall survival is being addressed in observational studies and emerging randomized clinical trials. Factors under evaluation include obesity and weight loss/maintenance, physical activity, dietary fat intake, and various dietary patterns. Studies have demonstrated that interventions targeting weight, diet, and physical activity lead to better quality of life and fewer disease and treatment-related side effects in breast cancer survivors [1–3]. While preliminary evidence suggests lifestyle factors can influence breast cancer incidence and outcome, validation studies are needed to support this concept [4, 5]. We summarize below the current evidence linking lifestyle factors and breast cancer incidence and outcome with emphasis on the findings from full-scale randomized trials and the status of ongoing randomized trials in this area.

72.1 Dietary Fat and Breast Cancer Incidence

Hypotheses were first proposed regarding a potential link between high dietary fat intake and obesity with breast cancer incidence and outcome about a half a century ago [6]. Despite the dozens of observational studies addressing the issue of lifestyle influence on breast cancer incidence and outcome, the findings have been mixed [4, 7–9]. In addition, level I evidence from the randomized clinical trial setting is still lacking. The need to obtain such evidence regarding the role of dietary fat intake on breast cancer incidence has been recently addressed in the Women's Health Initiative Dietary Modification (DM) Trial conducted at 40 US clinical centers [10]. Postmenopausal women between the ages of 50 and 79 years were eligible if they had no previous breast cancer, had a dietary fat intake >32% of total energy at baseline assessment, and additionally had a mammogram not suspicious for cancer. Body weight and height were serially measured. Mammogram screening was serially performed [11].

The dietary goal of the low-fat dietary program was to reduce fat intake to 20% of total energy and increase vegetable, fruit, and grain intake [12, 13]. Caloric restriction and weight loss were not targets of the intervention. The intervention was delivered by centrally trained nutritionists using a previously developed low-fat eating plan [13]. In the first year, there were 18 group visits with quarterly visits thereafter.

A total of 48,835 postmenopausal women were randomized. The intervention was successful in that dietary fat significantly decreased from 32% calories from fat to 20%, while fruit, vegetable, and grain intakes were significantly increased. However, the difference in fat intake between dietary and control group participants was somewhat less than called for in the study design. An additional methodology issue was that when the trial was designed in 1992, the time of the definitive analyses was based on a predetermined follow-up period, rather than on a specified number of events as conventionally done today. However, as full accrual took 1 year more than planned, the analyses were subsequently performed with about 1 year less follow-up. Of interest, although not an intervention target, women following a low-fat eating plan can lose weight, and there was a 3.2 pound, statistically significant difference in weight with lower weight seen in the dietary versus control group women and a difference maintained throughout the 8.3 years of dietary intervention [10].

When the study was reported at the end of the 8.3 years of dietary intervention, of the 1727 women diagnosed with invasive breast cancer, there were fewer cases seen in the dietary group (HR 0.91, 95% CI 0.83–1.01), but the difference was not statistically significant ($P = 0.07$) [10]. In subgroup analyses, women in the highest quartile of dietary fat intake at baseline experienced a statistically significant lower breast

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cancer incidence in the dietary comp group. Breast cancer mortality was also somewhat lower for women diagnosed in the dietary group with 27 versus 53 deaths, respectively (HR 0.77, 95% CI 0.48–1.22). However, the difference was not statistically significant. Despite the borderline results, this trial is considered negative and does not establish an influence of a low-fat dietary pattern on breast cancer incidence.

After the dietary intervention period ended, all contact with study nutritionists ended. There was interest to see if a statistically significant reduction in incidence would emerge with longer post intervention follow-up. However, with longer follow-up any signal for dietary effect on breast cancer incidence was lost [14]. Analyses suggesting a differential dietary effect during the dietary intervention period compared to the post intervention period have led to interest in conducting additional, secondary analyses focused on the breast cancer cases in the dietary and control group diagnosed during the dietary intervention period examining dietary influence on deaths from and after breast cancer including all 48,835 participants measured from randomization and breast cancer overall survival (breast cancer diagnosed during the dietary intervention period but followed through 2015) measured from diagnosis. These results, presented in abstract form, were suggestive of a favorable influence of the dietary intervention on breast cancer overall survival for cases diagnosed during the dietary intervention period [15]. These findings are currently undergoing peer review.

A second primary prevention trial entered 4690 women between the ages of 30 and 65 years with mammographic density >50%. Women were randomized to a lifestyle intervention designed to reduce fat intake to 15% of total calories and increase carbohydrates or to a control condition. A significant reduction in fat intake was seen. However, the dietary intervention had no influence on invasive breast cancer incidence (HR 1.19, 95% CI 0.81–1.55) [16].

72.2 Dietary Patterns Including the Mediterranean Diet and Breast Cancer

More recently, observational studies have examined the influence of various dietary patterns on breast cancer incidence and recurrence risk. A general consensus identifies several dietary patterns including western/unhealthy (incorporating high red processed meat, potatoes, sweets, high dairy) or prudent/healthy dietary patterns (high fruit and vegetable intake with poultry, fish, low-fat dairy, and whole grains). A Mediterranean dietary pattern generally follows a prudent guideline with an olive oil emphasis. Dietary quality scores have been developed to facilitate studies of associations with clinical outcome.

Studies examining associations between Mediterranean diets and breast cancer have provided mixed results [17–20]. The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort used the adaptive Mediterranean diet score (arMDS) [21] and found women with high adapted Mediterranean diet score had lower breast cancer risk (P = 0.048) [19], while other observational studies find no such associations [17, 20]. A review citing more recent studies identified five prospective cohort studies and eight case-control studies evaluating Mediterranean diet association with breast cancer risk. Pooled results identified a ten percent lower breast cancer incidence with higher Mediterranean diet intake in the case-control studies, but no association was seen in pooled results from the more reliable prospective cohort studies [22].

Despite such mixed results, interest in the Mediterranean diet and invasive breast cancer association was heightened by the results of secondary analyses performed in the Prevencion con Dieta Mediterranea (PREDIMED) study investigating a Mediterranean diet in a randomized trial in 4282 women aged 60–80 years at high cardiovascular disease risk. Participants were randomized to a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet "advised to reduce dietary fat." After 4.8 years of follow-up, there were 45 incident breast cancer cases with statistically significantly fewer cases seen in the Mediterranean diet with extra-virgin olive oil group (HR 0.32, 95% CI 0.13–0.79) [23]. While the control group was advised to reduce dietary fat, control group counseling was limited, and control group participants did not reduce fat intake substantially. While the numbers are small and this was a secondary analysis, this is the first randomized clinical trial demonstrating an effect of a dietary intervention on breast cancer incidence.

72.3 Comprehensive Lifestyle Behaviors and Breast Cancer

In addition to studies of specific nutrients and dietary patterns, recent studies have examined whether adherence to more comprehensive cancer prevention guidelines is associated with cancer incidence and outcome in prospective cohorts. In the Women's Health Initiative Observational study, the association between the American Cancer Society (ACS) nutrition and physical activity cancer prevention guideline score was associated with risk of incident cancer. The ACS cancer prevention guideline score includes four behavior-associated components: body weight, physical activity, diet, and alcohol consumption [24]. Behaviors least consistent with a recommendation were scored as 0, mid-level concordance were scored as 1, and behaviors that met criteria were scored as 2. While

smoking is not included in the ACS nutrition and physical activity cancer prevention guideline, it was nonetheless considered in a stratified analysis. With 8632 incidence cancers and 2356 cancer deaths, highest ACS guideline scores were associated with a 17% lower risk of any cancer, 22% lower risk of breast cancer, 52% lower risk of colorectal cancer, and 27% lower risk of all-cause mortality with all findings statistically significant [25].

The World Cancer Research Fund/American Association for Cancer Research (WCRF/AACR) published eight nutrition-related recommendations for cancer prevention. Adherence to these recommendations was investigated for association with breast cancer incidence, overall survival, and by hormone receptor subtype in the Swedish Mammogram Cohort of 31,514 primary postmenopausal women [26]. With a score based on adherence to recommendations for body weight, physical activity, energy density, plant foods, animal foods, alcohol drinks, and dietary supplements for a score ranging from 0 to 7, during 15 years of follow-up, women meeting 6–7 recommendations had a 51% lower risk of breast cancer compared to those meeting two or less recommendations (95% CI 0.35–0.70). The association was strongest for estrogen receptor-positive/progestin receptor-positive subtypes, while estrogen receptor-negative/progestin receptor-negative subtypes were associated with adherence to recommendations regarding plant and animal food intakes. Taken together, such reports provide a sound basis for intervention strategies targeting a comprehensive range of recommendations rather than focus on individual nutrients.

72.4 Breast Cancer and Obesity

Obesity is a major health problem with over 68% of adult women in the USA currently being overweight or obese [27]. Obesity has been consistently associated with higher breast cancer risk in systematic reviews and meta-analyses of observational studies with about 20% higher breast cancer incidence in obese women compared to women with normal weight [28]. Despite these strong associations, observational studies have largely failed to establish that adult weight loss of overweight or obese women will reduce breast cancer incidence. In recent analyses in the Women's Health Initiative (WHI), this question was revisited in secondary analyses of over 67,000 postmenopausal women participating in the WHI clinical trials [29]. Women who were overweight and obese had a higher breast cancer risk. For women with obesity class II or III (BMI >35), the hazard ratio for invasive breast cancer was HR 1.58, 95% CI 1.40–1.79. The risk was even higher for hormone receptor-positive breast cancer (HR 1.86, 95% CI 1.60–2.17), but no association with hormone receptor-negative cancers was seen. The effect of weight change during the 13 years of follow-up on breast cancer was examined in women with normal weight (BMI <25.0); those who increased their body weight by more than 5% had increased breast cancer risk compared to women who maintained their body weight (HR 1.36, 95% CI 1.10–1.65). However, in women already overweight or obese, those who decreased their body weight by more than 5% experienced no decrease in their breast cancer risk [29]. While there are other health benefits associated with weight loss for overweight or obese adult women, given current evidence, it is not possible to provide a strong public health message regarding breast cancer risk reduction benefit of weight loss for overweight or obese women.

One issue complicating evaluation of the association between weight loss and breast cancer risk is the issue of voluntary as compared to involuntary weight loss. In postmenopausal women, about one third of weight loss is involuntary. This involuntary weight loss, likely associated with other health problems, may confound analyses attempting to associate weight loss with lower breast cancer risk. Information on voluntary and involuntary weight loss has been captured in over 91,000 postmenopausal women participating in the WHI observational study. In this population, the issue of voluntary weight loss influence on subsequent breast cancer risk is being prospectively addressed.

72.5 Dietary Fat and Breast Cancer Recurrence

There has been ongoing interest in determining whether a low-fat dietary pattern could favorably influence breast cancer clinical outcome (recurrence and overall survival) of postmenopausal women with early-stage disease receiving standard adjuvant therapy. After feasibility of achieving dietary fat intake reduction in the post-diagnosis setting in a six-institution randomized trial involving 300 patients was demonstrated [30], Dr. Ernst Wynder, one of the first scientists to propose the dietary fat hypothesis, led a multicenter, randomized controlled clinical trial to evaluate whether a dietary program designed to reduce fat intake could influence breast cancer recurrence risk. A total of 2427 postmenopausal women entered the study, and during the 5-year median intervention, fat intake was reduced from 38% to 25% calories from fat ($P < 0.0001$), and body weight was reduced as well (−6.0 pounds [mean], $P = 0.005$). Relapse-free survival was the primary study endpoint and was increased by the dietary intervention with fewer relapse-free survival events seen (HR 0.76, 95% CI 0.60–0.98, $P = 0.03$ from the adjusted Cox proportional hazard model) [31].

Funding issues precluded ongoing active follow-up after the intervention ended. A report based on incomplete follow-up found the dietary influence on breast cancer incidence

fading once intervention ended. Of interest was a subgroup analysis (not protocol mandated) that found women with estrogen receptor- and progestin receptor-negative cancers had significantly greater survival in the dietary group (HR 0.36, 95% CI 0.18–0.74, $P = 0.0003$) [32].

A second phase III study evaluating dietary change and breast cancer outcome is the Women's Healthy Eating and Living (WHEL) trial. The study examined a multicomponent dietary intervention targeting increase in vegetable servings, 16 ounces daily vegetable juice, increase in fruit and fiber intake, and a target of reducing fat intake to 15–20% of calories. While fruit and vegetable intake were substantially increased, no sustained decrease in percent energy from fat was seen with intake of fat quite similar at 6 years of follow-up. No suggestion of a dietary effect was seen with breast cancer event-free survival of HR 0.96 (95% CI 0.81–1.14, $P = 0.63$) [33].

While there are several differences between the WINS and WHEL trials in terms of eligibility, study design, and dietary intervention focus [34] (Table 72.1), a hypothesis that emerged from the WINS experience was that a lifestyle change promoting a low-fat dietary pattern which is associated with weight loss could have potential influence on breast cancer recurrence risk. Obviously, such a hypothesis requires verification in a randomized trial. The importance of moving forward with clinical trials providing definitive evidence regarding energy balance interventions to reduce cancer morbidity and mortality has been recently recognized in an American Society of Clinical Oncology statement [35].

72.6 Ongoing Full-Scale Studies of Lifestyle Change and Breast Cancer Outcome

The effect of lifestyle intervention on breast cancer recurrence risk is currently under evaluation in several large randomized prospective clinical trials. The SUCCESS C is a European-based trial evaluating increased physical activity and weight loss/maintenance using a factorial design, implemented in a randomized clinical trial setting where several taxane regimens are under evaluation. As only about 1000 patients are anticipated to be randomized, only relatively large effects of the lifestyle intervention will be detectable

Table 72.1 Completed and ongoing clinical trials evaluating lifestyle interventions as addition to adjuvant breast cancer management

	WINS[a]	WHEL[b]	SUCCESS C	DIANA-5
Eligibility				
Stage	I–III A	I–II A	Node positive, high risk, node negative	High risk[c]
Time from surgery	≤12 months	≤48 months	At diagnosis	<5 years
Age	48–79 years	18–70 years	Pre- and postmenopausal	35–70
Diet at baseline	≥20% calories from fat	Any	Any	Any
Dietary intervention	Individual sessions with dietician	Telephone-based sessions	Telephone calls from lifestyle coach	Cooking classes, conferences, exercise sessions
Number of patients	2437 (3:2 randomization)	3088 (1:1 randomization)	1000 (estimate) (1:1 randomization)	1214 (1:1 randomization)
Intervention targets				
Fat	↓ to 15% calories from fat	↓ to <20% calories from fat	↓ to 20–25% calories from fat	Mediterranean macrobiotic diet
Vegetable	Increase (no target)	Increase to five servings and 16 oz vegetable juice/day	Increase (no target)	See above
Fruit	Increase (no target)	Increase to three servings/day	Increase (no target)	See above
Body weight	N/A	N/A	Weight loss target	Weight loss target
Physical activity	N/A	N/A	↑ to 150–200 min moderate PA/week	↑ to 210 min moderate PA/week
Endpoint	Relapse-free survival	Breast cancer event-free survival	Breast cancer recurrence	Breast cancer events
Primary breast cancer outcome	HR 0.76 (95% CI 0.60–0.98, $P = .63$)	0.96 (95% CI 0.80–1.14, $P = .63$)	Pending	Pending

[a]Women's Interventional Nutrition Study
[b]Women's Healthy Eating and Living Study
[c]ER-negative or metabolic syndrome on high testosterone or insulin

[36]. The DIANA-5 is another ongoing trial evaluating a lifestyle intervention evaluating a Mediterranean diet and increased physical activity influence on breast cancer recurrence risk compared to a control condition. All participants will receive standard breast cancer therapy as clinically indicated [37]. The study has assigned 1208 breast cancer patients between 35 and 70 years of age with early-stage disease to the lifestyle change or control conditions. Follow-up is anticipated to be completed in 2015. Again, the sample size will only allow a large difference between randomization groups to be detected. The design features and current status of completed and ongoing adjuvant trials evaluating lifestyle interventions are described in Table 72.1.

A full-scale, randomized, controlled clinical trial evaluating a lifestyle intervention in clinical centers in North America is scheduled to begin in 2016 led by Dr. Jennifer Ligibel. The trial will enter 3500 early-stage breast cancer patients who will be randomly assigned to a lifestyle intervention targeting increased physical activity and weight loss/maintenance or a control condition. All will receive standard adjuvant chemotherapy, endocrine therapy, and radiation therapy as indicated by their disease stage and disease characteristics. The centrally mediated, telephone-based intervention has had demonstrated efficacy in a multi-institution feasibility study involving over 300 breast cancer patients where the intervention was successful in increasing physical activity and decreasing/maintaining body weight [38, 39]. A full-scale adjuvant breast cancer trial with similar lifestyle targets is proposed by the Institut Catala d'Oncologia in Spain led by Dr. Antonio Agudo. The plan is to recruit 2000 early-stage patients to a randomized trial evaluating the effects of weight control, diet change, and physical activity intervention on breast cancer recurrence.

72.7 Mechanisms of Action

Several biological models outlining potential mediating factors related to obesity and dietary pattern influence on breast cancer risk include insulin, estrogen, and markers of inflammation [4, 40, 41]. A number of relatively small trials have associated several lifestyle interventions with modest changes in these proposed mediating factors. While such work is important, we have a lesson from the tobacco and cancer experience which perhaps has relevance for study of lifestyle and breast cancer as well. For over a quarter-century, there was a concerted effort to precisely define the mechanism by which tobacco mediated the large increase in lung cancer associated with its use. However, when the focus shifted to the public health setting with development and implementation of policies and procedures designed to reduce smoking in the general population, reductions in tobacco exposure with resultant reduction in cancer risk have been seen. Likewise with lifestyle, wide-scale implementation of programs to influence favorable lifestyle choices based on influence on other diseases could well impact breast cancer incidence and outcome as well.

72.8 Physical Activity

The lifestyle factor most consistently associated with both breast cancer incidence and breast cancer outcome is physical activity [4]. A recent meta-analysis of observational studies found higher physical activity levels associated with lower breast cancer incidence in both pre- and postmenopausal women (RR 0.80, 95% CI 0.78–0.84) [42]. In terms of physical activity and subsequent breast cancer outcome, a meta-analysis from 22 prospective cohort studies was conducted with 123,574 participants and 6898 all-cause deaths [43]. Compared to those who reported low/no physical activity, high post-diagnosis physical activity was associated with fewer all-cause deaths (HR +0.52, 95% CI 0.43–0.64, $P < 0.01$) and fewer breast cancer-related deaths (HR = 0.59, 95% CI 0.45–0.78, $P < 0.05$).

Of most relevance to the management of breast cancer are studies which compare the timing of the physical activity in relation to the cancer diagnosis. Such studies directly address the clinically relevant question of whether a woman with higher physical activity after diagnosis can reduce her recurrence risk regardless of her physical activity before diagnosis. In a report from the Nurses' Health Study, 2987 breast cancer patients with early-stage disease provided self-report of physical activity prior to diagnosis (retrospectively) and about 2 years after diagnosis. Physical activity over 9 MET/h per week was associated with lower recurrence risk [44]. In a report from the Women's Health Initiative, information on physical activity was prospectively collected both before and after breast cancer diagnosis in 2076 early-stage patients. Breast cancer deaths were lower only in women who maintained an active physical activity pattern or who increased physical activity after diagnosis but not in those who are inactive in both periods or who were previously active but decreased their activity post-diagnosis [45–49]. Most recently, a prospective pooling study examined this issue in 6295 estrogen receptor-positive early breast cancer patients. Pooled and harmonized data were available on clinical and lifestyle factors adjusting for clinical factors; post physical activity was inversely associated with 5-year all-cause mortality (HR 0.81, 95% CI 0.71–0.93) (4.92 < 17.4 MET/h/week) [50].

Several moderately sized randomized trials have successfully evaluated a number of strategies to implement physical activity increase in breast cancer survivors [3, 51–53]. The ongoing full-scale trials attempting to confirm the findings from observational study reports are described elsewhere in this report.

72.9 Pragmatic Clinical Trials Evaluating Lifestyle Interventions

Pragmatic trials provide an emerging avenue for evaluation and broad implementation of medical strategies. An example of a pragmatic clinical trial involving a lifestyle intervention is the ongoing Women's Health Initiative Strong and Healthy (WHISH) trial led by Dr. Marcia Stefanick. The trial will evaluate physical activity increase with a goal of 4 h/week walking equivalent compared to a control condition on cardiovascular disease and cancer in postmenopausal women 65 years of age or older. As these women had already been consented for medical outcome follow-up as part of the WHI cohort, the recruitment was done by mail, and, following National Institutes of Health Guidance, the consent was not required from controls. In a 6-month period, the full study complement of 50,000 postmenopausal women has been randomized and the intervention is ongoing. Such studies provide an efficient and cost-effective method of evaluating lifestyle influences on chronic disease in postmenopausal women. In addition, upon determination of favorable influence on chronic disease outcomes, such a strategy provides a cost-effective method of broadly implementing a lifestyle intervention especially in individuals participating in an integrated healthcare system.

72.10 Summary

While there is much evidence from observational studies that lifestyle factors can influence breast cancer incidence and outcome, findings in such studies have limitations. An editorial by Dr. Pamela Goodwin [54] focusing on obesity outlines circumstances which could account for a noncausal association between obesity and increased risk of breast cancer recurrence such as lower compliance to cancer therapy or physician decision to reduce chemotherapy dose in obese women. Even if an effect of obesity is causally related to the adverse outcome, the effect may not be reversible. For example, in the WHI dietary modification trial, there were more progestin receptor-negative cancers in the control compared to the dietary intervention group [10]. Progesterone receptor-negative cancers have a worse prognosis, and it is not clear that changing one's dietary intake after diagnosis could reverse the finding. That is, the higher dietary fat intake may have led to a fixed adverse prognosis that is independent of subsequent lifestyle change. Thus, favorable associations with certain lifestyle practice and breast cancer incidence and outcome must be both causal and reversible to inform medical practice and public policy (Table 72.2).

Table 72.2 Obesity and breast cancer outcome: possible explanations for an association

Type of association	Effect on outcome
Noncausal	Healthy person bias, e.g., normal weight women, are destined to do better, possibly due to greater compliance, treatment tolerance, or adoption of healthy behaviors
	Less aggressive treatment of obese patients
	Presentation of breast cancer at a more advanced stage in obese women
	Other biases or confounders
Causal	Nonreversible: Obesity has affected the type of cancer that has developed, leading to a fixed biologic effect
	Reversible: Obesity affects cancer on an ongoing basis, and this impact can be reversed when weight loss occurs

Note: Weight loss would be postulated to lead to improved cancer outcomes (e.g., decreased risk of recurrence or death) only if the association is both causal and reversible
From Goodwin [54]

While ongoing randomized clinical trials are designed to provide highest level of evidence supporting lifestyle influence on breast cancer outcome, there are established other health benefits for weight loss/maintenance and higher levels of physical activity. Also, the current lifestyle strategies being evaluated, namely, increase/maintenance of 4 hours of walking equivalent per week and maintenance of normal weight or targeting 5% weight loss if overweight or obese, are achievable by many with no/little toxicity and low cost. As current evidence is suggestive of benefit of such lifestyle strategies on breast cancer outcome, while awaiting randomized clinical trial evidence, women with breast cancer should consider incorporating these activities into their daily routine. The recent American Cancer Society/American Society of Clinical Oncology Breast Cancer Survivorship Care Guideline endorses such an approach (Table 72.3) [55]. Physicians managing breast cancer patients should discuss the current level of evidence, including the limitations of such evidence, associating lifestyle decisions with more favorable breast cancer outcome.

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Table 72.3 ACS/ASCO Breast Cancer Survivorship Care Guideline recommendations for obesity, physical activity, and nutrition

	Level of evidence
Obesity	
(a) Should counsel survivors to achieve and maintain a healthy weight	0 (maintenance)
(b) Should counsel survivors if overweight or obese to limit consumption of high-calorie foods and beverages and increase physical activity to promote and maintain weight loss	IA, III (weight loss)
Physical activity	
Should counsel survivors to engage in regular physical activity consistent with the ACS guideline and specifically:	
(a) Should avoid inactivity and return to normal daily activities as soon as possible following diagnosis	III (avoid inactivity)
(b) Should aim for at least 150 min of moderate or 75 min of vigorous aerobic exercise per week	I, IA (aerobic exercise)
(c) Should include strength training exercises at least 2 days/week; emphasize strength training for women treated with adjuvant chemotherapy or hormone therapy	IA (strength training)
Nutrition	
Should counsel survivors to achieve a dietary pattern that is high in vegetables, fruits, whole grains, and legumes Low in saturated fats and limited in alcohol consumption	IA, III (nutrition); 0 (alcohol)

From Runowicz et al. [55]

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Additional information: A full list of all the investigators who have contributed to Women's Health Initiative science appears at https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

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Psychological Support in Breast Cancer Patients: A Personalized Approach

73

Chiara Fioretti, Ketti Mazzocco, and Gabriella Pravettoni

It is widely recognized that a breast cancer diagnosis generates a biographic disruption in a woman [1]. The discovery of an insidious disease such as breast cancer is most of the time experienced as a life interruption creating a large gap between the life before and after the diagnosis. In this perspective, the main goal in patient's life, together with fighting the disease, becomes re-establishing the natural life balance.

This process of reconstruction is not exempt from psychological and emotional suffering. The most recent literature on the psychological consequences of cancer disease reveals that one in three patients meets the criteria for mental disorders [2]. Exploring the 4-week prevalence of mental disorders in a total sample of 5889 German patients, Mehnert and colleagues found that the prevalence for any disorder was 31.8%, including anxiety disorder (11.5%), adjustment disorder (11.1%), mood disorder (6.5%), somatoform disorder (5.3%) and others [2].

These results highlight the strong need for a psychological intervention in cancer patients, especially because of the higher 12-month mental disorder prevalence in this population (18.4%) compared to the general population (13.3%) [3], with the highest prevalence (41.6%) in breast cancer [2].

In line with these findings, Arnaboldi et al. [4] investigated the post-traumatic stress disorder (PTSD) symptoms as a consequence of breast cancer disease in the first 30 days after diagnosis and how the symptoms changed over time. Results showed that psychological symptoms in 30 days after diagnosis were anxiety (70.7%), intrusion thoughts (20%) and avoidance thinking and behaviour (19.1%). Particularly, anxiety varied over time, with a pick (76.4%) at the moment of the pre-hospital admission.

Looking at these epidemiologic data, breast cancer has important clinical implications in patients' lives: the diagnosis and the first contact with the hospital context are experienced as a traumatic event by a high percentage of women.

73.1 Supporting the Psychological Distress in Breast Cancer Patients

The psychological distress related to breast cancer is not just a matter of mental disorders. Even the patients who do not experience such disorders can feel a strong distress related to the discovery of being affected by a serious disease.

Cancer-related distress has been defined as "a psychological (cognitive, behavioural, emotional), social, and/or spiritual state that may interfere with the ability to cope effectively with cancer, its physical symptoms, and its treatment" [5]. Supporting patients' distress means to take into account their symptoms from a multifactorial point of view: physical, social and emotional. In breast cancer patients, distress is often related not only to physical symptoms but even to the personal body image perception that, together with treatments, have relational and sexual implications. For example, the hormonal alterations caused by endocrine therapies and chemotherapy direct consequences on the physiology of desire and sexual response [6]. Regarding surgical treatments, mastectomy, quadrantectomy and the more recent nipple-sparing mastectomy importantly affect body image. Thus, even though breast surgery is becoming increasingly conservative with a positive impact on psychological adjustment to disease, body image and sexuality [7], the emotional reactions to cancer, physical signs as scars and the type of treatment can still be detrimental for sexual life. In addition, side effects of medical treatments increase the patient's worries and negative emotional reactions towards her condition. Furthermore, the disruption of ovarian function and menopause due to endocrine modulators are other crucial side effects [8, 9] making pregnancy impossible [10]. The impact of this in young women

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cannot always be predicted since it is strongly influenced by individual hormonal profiles and by age. However, even postmenopausal women seem to face sexual difficulties due to the effect of medical therapies, including those involving the adjuvant use of aromatase inhibitors for the treatment of early breast cancer [11].

In general, women tend to feel worries about the sexual encounter with their partner. Any impairment in breast shape and in breast sensitivity demolishes personal certainty and casts the shadow of a doubt over whether the partner will accept these changes.

Nevertheless, in women, the breast is not just part of the sexual life and the relation with the partner, but it acquires meanings due to its generative and nutritional features. The breast, in such a way, is a vehicle of life, the symbol of a femininity which is not only related to a personal image but also to the natural, social and cultural function of generating and feeding. This issue is much more evident in younger women. In fact, the increase in women's awareness of the importance of periodic breast examination, together with the improved accuracy of imaging technology, has considerably increased the number of young women of reproductive age diagnosed with breast cancer. According to these aspects, it is important to understand the specific meanings that each breast cancer woman ascribes to the disease and its consequences in order to uncover the cognitive determinants of emotional reactions and behavioural responses. In this meaning attribution process, to better tailor communication and therapeutic plans, professionals should not ignore partner's meanings associated to the disease.

In a study of Piot-Ziegler and colleagues [12], breast cancer patients described mastectomy, hair loss and other disease consequences as "threat to their body integrity", "a stigmatization" as well as an insult to their femininity. Breast removal and other body changes can bring a destabilization in patients' perception of their own bodies, negatively affecting (Piot-Ziegler et al. [12]) the establishment and the maintenance of a good social network.

In this scenario, patients' quality of life could be damaged, and scientific evidence has stressed that adopting a multidisciplinary team is the best way to re-establish it again. In this sense, professionals should adopt a biopsychosocial approach, caring for patients considering them as people acting in a context of constant changes and adaptation to the physical, relational and cultural environment [13]. In this perspective, the patient has to be considered as a system in which different components cooperate to shape an organizational entity with specific functions. Thus, physical conditions have to be taken into account together with the emotional and relational ones. At the same time, patients' needs cannot be separated from their caregivers' ones.

In a biopsychosocial point of view, the same experience of pain, for instance, can be perceived as the result of the interaction among physical, psychological and social factors. Biological factors produce the pain sensation, psychological factors contribute to the pain perception and to the cognitive and semantic consciousness, and finally social factors influence the individuals' reactions and behaviours in facing pain.

Collecting information on the different components of patient's experience is a way to improve both doctor-patient relationship and patients' clinical outcomes, increasing their adherence to treatments [14]. In fact, the link between psycho-emotional suffering and organic disease development has been amply revealed in scientific literature [15]. In the biopsychosocial model, communication is a powerful tool to investigate and deepen patient's needs. It is not just useful for collecting the patient's story but also for constructing a therapeutic path together with patients, adopting a shared decision-making process which takes into account their preferences, needs and habits.

In this domain, the subjective experience of breast cancer distress requires a specific psychological support in any different phases of the disease, from initial diagnosis to after the completion of cancer treatment.

73.2 Supporting the Diagnosis Phase: Improving Patient Empowerment

As we stressed in the first part of this chapter, the diagnosis of breast cancer is experienced most of the times as a biographic disruption by the patient [1]. Scientific evidence underlines that this phase is characterized by the highest prevalence of distress and PTSD symptoms [4]. In this sense, the psychological support in this delicate phase should aim to help the patient to manage the bad news and find resources to face the life fragmentation.

Although high state anxiety and depressive symptoms are important problems in women during and after the diagnostic process for breast disease, in many women, they can be considered as a momentary emotional condition characterized by natural subjective feelings of apprehension and tension. One of the most common errors that health professionals make is considering all the psychological natural reactions to the diagnosis as a sign of mental disorder. Sentences such as "that patient is depressed" are quite commonly used by the professional staff talking about a new breast cancer patient. From a psychological point of view, feelings of anxiety or mood swing can be considered as operational reactions to the diagnosis communication. As professionals, from a biopsychosocial point of view, it is necessary to take into account what the patient is communicating to us: she is feeling distress and she is reacting against the new condition of disease. Our role in supporting this phase is to help the patient to find the best individual resources considering her personal characteristics (personality traits, age, life goals, economic resources) and social and cultural support (significant relationships, presence of children, job position).

In this scenario of deep fragmentation and readjustment after the diagnosis, the patient is also involved in important decisions to make with her oncologist.

Box 73.1 Different experiences in decision-making on breast cancer surgery

The case of L.	The case of M.
L. is a beautiful 30 years old woman that came to the *** hospital for an oncological consultation after the diagnosis of cancer at her right breast. L. comes from a beautiful town in central Italy and is accompanied by her boyfriend. She affirms to be shocked because of her diagnosis: she's a medical student and she has always been very scrupulous with her health. In the last month after the bad new, she has visited several professionals searching for the best therapeutic option. Now she has two possibilities: the most advantageous from the oncologist point of view seems to be the mastectomy. The intervention is invasive but she will not need other therapies. The second option is a quadrantectomy: it will be less invasive and her mammary gland will be save, but she will need radiotherapy to complete the treatments. L. has a decisional dilemma: she's young and one of her main expectations from the future is to become mother. Furthermore, she has a very good relationship with her boyfriend and just before the diagnosis they were planning to get married. She has to decide between keep her breast going through heavy therapies or lose her breast undergoing a mastectomy.	M. is a 45 years old woman that came with her husband to the *** hospital after having received the diagnosis of mammary carcinoma at her right breast. After the bad new, M. decided to do the genetic test to investigate the presence of BRCA1 and 2 mutations. The test is negative. M. reveals to have been shaken for the diagnosis, but she is not new to this kind of disease. For several years she experienced this terrible disease while taking care of her best friend, who recently died for a breast cancer. She affirms to be not strong enough to face a new suffering. Although her oncologist has suggested her the opportunity to undergo a quadrantectomy considering her young age and her medical conditions, she refers to be absolutely sure that the mastectomy is the best option for her. She wants to reduce the risk of a second carcinoma and she asks for a mastectomy intervention. Furthermore, she tells that her breast has always been a problem for her: since puberty she felt pain and lack of self-confidence, especially in the sexual relationship with her husband. For this reason she also refused to breastfeed her two kids.

As we can see in the cases of L. and M. (see Box 73.1), every patient brings her own life story in medical decisions, and health professionals have to take it into account in defining and proposing the therapeutic path.

Although the two women apparently experienced a similar diagnosis, their personal and social characteristics have a great influence on their decision about the therapeutic path. L. is very young, and she is planning her life with the boyfriend. One of her main dreams is to have a baby. The fear for the physical consequences of therapies on her fertility is strong. On the other side, she would prefer to have a quadrantectomy saving her breast. On the contrary, M. appears very certain about her choice: she suffered for the recent loss of her best friend, and she wants to reduce as much as possible the risk of a second carcinoma opting for a mastectomy. Which is the most functional response that health staff can act to help the patient in this difficult choice?

During the decisional phase, the communication between the patient and the physician is a critical point: information given by all professionals involved in the care (surgeons, oncologists, nurses) have the aim to provide a clear description of the options and to facilitate the patient's accurate evaluation process which leads to realistic expectations on the final result [16].

In other words, professionals need to empower patients' role in the disease management.

Patient empowerment is defined as a social process in dealing with recognizing, promoting and enhancing peoples' abilities to meet their own needs, solve their own problems and mobilize necessary resources to take control of their own lives [17]. It refers to the need of actively involve patients in

treatment decision-making in order to promote decisions that are consistent with their values, preferences and daily life management possibilities. This shared decision-making process empowers the patient because it provides him/her with the chance of making his/her own well-discussed and well-informed choice concerning the treatment. In the specific case of breast cancer, the nature of malignant disease requires women to make difficult decisions regarding ensuing treatment, as we have seen in the L. and M. cases. Scientific evidence underlines that having relevant information not only helps cancer patients to understand the disease, but it also facilitates their decision-making and coping with the disease [18, 19].

Furthermore, patient empowerment is also a powerful instrument to face distress and life fragmentation: guided from the health staff, the patient will learn to manage information on the disease and to use them to make the best decision for their own life. This active role will provide the woman for new expectation for the future and new life goals that take into account the present condition of disease.

In this decisional context, the patient has to analyse benefits and risks of received information in short and long term, as well as the changes in her life style requested to implement the therapeutic path. The right choice seems to depend on both clinical and individual needs, as well as on patient's expectations and cognitive traits. Every woman has her own decisional style, defined as "the methodical inclination that the individual has when they face a decision making" [20]. Knowing the patient's decisional style can help professional in understanding how to conduct the encounter with the patient and the decision-making process. This will be a way to increase therapeutic alliance and the patient's adherence to the treatment.

73.3 Accompanying Patients in the Treatment Phase of the Disease: A Personalized Approach

For the majority of women facing a breast cancer, the treatment phase starts with breast surgery. As we have seen in the case of L., the two prevalent options are breast conservation surgery (with radiation therapy) and mastectomy with or without reconstruction [21]. Although both the interventions affect women's breast and body image, the psychological implication can be very different. Many women perceive mastectomy as a mutilation damaging both their body and their femininity. The psychological support, in this case, should aim to help the patient to accept the loss and the new body image. Furthermore, with the breast reconstruction, women experience a strong reduction of breast sensibility, with consequences on their intimacy and sexual relation with the partner. On the contrary, local tumour removal appears the best option in the case of early-stage breast cancer [21]. Beside the low invasiveness of conservative surgery in local tumors, several consequences are associated with the adiuvant radiation therapy, that is often needed after such a surgery: fatigue, skin changes, pain, psychological distress and interferences in daily functions [22].

Breast cancer patients are often involved in adjuvant systemic therapies causing several physical and neuropsychological symptoms [21] as pain, fatigue, nausea and vomiting, sexual problems, cognitive dysfunctions, sleep disorders, weight and appetite disorders. Scientific evidence has highlighted that reduced quality of life entails that a significant fraction of breast cancer patients is noncompliant with adjuvant therapies [23, 24].

Psychological support in this scenario strives for improving patients' decreased quality of life starting from the personal needs. In this sense, the treatment phase of disease needs a personalized approach [25, 26]: the main aim of health professionals is that of enabling patients to participate and guide their own healthcare, increasing their autonomy and self-determination. Studies assessing patients' wishes within a personalized medicine framework have underlined their need to have adequate information and permission to participate in decisions which affect them, as well as the request for receiving such information with empathy, dignity and respect, taking into account their preferences and their personal social characteristics [25]. Furthermore, studies demonstrated that when this kind of wishes is embraced, patients have better health outcomes, higher adherence to treatments and increased trust in health professionals [27]. According to the most recent approaches in medicine, therapies have to be tailored on the basis of both the biological and the molecular characteristics of the disease without ignoring the psychological and cognitive characteristics of the patients [28]. "The way in which each patient reacts to his/her illness, understands his/her clinical condition, forms an opinion about possible treatments, adheres to treatments, copes with treatment side effects, and interacts with the whole health care process adds new dimensions to human uniqueness in the same way that genetic information does" ([25], p. 685).

Other medical approaches, such as narrative medicine [29], point out the importance of considering the patients' individual life story in identifying the best therapeutic option for every specific patient [30].

In M.'s point of view, for instance, the psychological implications and the wish to reduce the risk of a secondary carcinoma are stronger than the physical and emotional impact of mastectomy. In supporting the decision-making process, oncologists have to consider M. personal characteristics: she is 45 and a mother of two children and she never felt comfortable with her breast. Although the clinical conditions would suggest the opportunity to opt for a breast con-

servation surgery, M. feels that mastectomy and reconstructive surgery are the best option for her.

Patients' psycho-cognitive traits also need to be considered by defining a personal profile of his/her specific needs and values, habits and behaviours, hopes and fears and beliefs [25].

Recently, the European Community has promoted research projects (www.p-medicine.eu) aiming to investigate the impact of personalized medicine on doctors' and patients' disease management. Questionnaires such as ALGA-C [28] have been validated in the field of breast cancer to obtain a patient's profile useful to help physicians achieve meaningful personalized care which supplements biological and genetic analysis and therapies.

73.4 After the Storm: Re-establishing the Life Balance

Substantial progress has been made in the early detection, diagnosis and treatment of cancer in the last two decades. More and more patients can now manage their disease as a chronic illness requiring long-term surveillance and, in some cases, maintenance treatment that stretches from prevention to the end of life.

Chronic cancer treatment places new demands on patients and families to manage their own care. On the other side, physicians need to manage long relationships with their patients, adjusting their care to the specific need of the patient in any phase of her life. In fact, patients undergo periodical treatments and tests, and the transition from treatments to survivorship is not exempt from emotional distress, feeling of anxiety and uncertainty [21].

Although studies highlight that quality of life improves for the majority of women [21], the need for being accompanied in this delicate transition is strong. This is the phase in which patients start to reintegrate themselves in their daily lives. In this sense, psychologists and health professionals have to encourage the retake of daily activities such as job and family activities, helping women to accept those changes that the disease inevitably brought in their lives. Once again, the therapeutic alliance has to focus on a personalized approach. If some cognitive and psychological patients' characteristics suggest to encourage a rapid separation from the "hospital world", other patients would need to be gradually accompanied in this process.

Scholars have found also that breast cancer survivors' memory and learning performance are significantly lower than the healthy control. Particularly, women undergoing endocrine therapy have weakness in initial encoding of information into working memory [31]. In the relationship with their patients, professionals have to inform about cognitive impairments due to hormonal therapies. In this sense, psycho-oncologists can address recommendations on cognitive rehabilitation strategies and help the patient to find compensatory strategies. In particular, it is important to limit distractions and multi-tasking at the time of learning, to suggest semantic or visualization strategies that emphasize deeper information processing levels, to reduce environmental cognitive load and to educate to self-monitoring for inattention and distraction [31].

In this transition to the survivorship, women could also need a support in the reconstruction of their routine with significant others: children, partners and parents. Caregivers' involvement in this transition is warmly recommended.

Despite the progress in diagnosis and treatment of breast cancer, many women face a disease progression and go through the transition from the active treatment to the palliative care. In this case, the psycho-oncological support has to take into account the individual need of every woman. The patients' emotional distress has to deeply consider in order to evaluate the boundary between an existential suffering due to the poor prognosis and a psychological disorders such as anxiety or depression [21]. Once again, a personalized approach will lead professionals to help the patient to focus on specific goals about quality of life, pain management and relationship with significant others, starting from the personal needs.

73.5 New Challenges in the Psychological Support of Breast Cancer

73.5.1 Managing Genetic Risk Information

Protocols and clinical indications in the field of breast cancer therapies are constantly developing. Similarly, psychological support in breast cancer has to accept the challenge and provide for the integration of new patients' needs in theoretical and intervention models.

Undoubtedly, one of the big advances in breast cancer diagnosis has been the identification of BRCA1 and BRCA2 mutation: researchers have found that approximately 10% of breast cancers are due to hereditary disposition. Breast cancer patients and healthy women have the opportunity to undergo genetic counselling to receive information on their cancer risk and psycho-decisional support to be guided in the evaluation of the available treatment options, their risks, costs, benefits and personal values and priorities.

Although identifying individual risk factors may allow the development of personalized interventions decreasing the probability of disease development, not all women are "information seekers", and some of them could prefer to not undergo genetic testing. Risk perception is extremely subjective and can depend on several factors as familiar story knowledge, patient activation, family communication issue, trust in medical sciences and physicians, personal cognitive

and thinking styles. Furthermore, studies identified copious factors predicting psychosocial distress in genetic testing process [21]. Among these, the experience of cancer in a family member seems to be a high-risk factor for emotional distress, as well as psychological traits as coping style and resilience and socio-demographic factors, such as having children.

In this domain, genetic testing protocols should involve psychological support across all the decisional phases. Psycho-decisional support should aim to investigate woman's decisional process focusing on individual and familiar issues.

We recommend to involve psychologists in genetic counselling in at least two phases of the decision-making process: when the woman is proposed to undergo genetic testing and when she receives test results.

73.5.2 Breast Cancer and Pregnancy

A second current big challenge in the field of breast cancer treatment and psychological support is the management of cancer diagnosed during pregnancy. The rate of breast cancer diagnosed during pregnancy, although rare, has increased in the last years also because of the continuous growth of the age of women pregnancy [32]. In this sense, the main goal of professionals and researchers in the field is to provide treatments as similar as possible to that offered to nonpregnant young patients with breast cancer.

In the delicate transition to motherhood, Henry et al. [33] found that physiological distress due to pregnancy is prone to become long-term distress in breast cancer patients because of the several worries and fears related with a cancer diagnosis.

When providing psychological support in breast cancer patients during pregnancy, many factors have to be considered. Professionals and patients face a big number of decisional dilemmas that have to be investigated starting from the individual needs of every woman. Among these, scholars and clinicians highlight the need of evaluating a hypothetic pregnancy interruption, the selection of the best surgery and the timing for breast reconstruction, the possibility of the sentinel lymph node biopsy as well as the decision about following therapies.

Although scientific evidence underlines that abortion does not improve survival of breast cancer patients [34], termination of pregnancy is often suggested, and women are asked to deal with the quandary of deciding between the pursuit of their own life and that of their children [33].

In defining treatment and surgery options, the gestational age is the most important factor to take into account [35]: in the first trimester, exposition to radiations and treatments has potential critical effects on the foetus that lead to not recommend them. Similarly, professionals suggest the delay of treatments in patients very close to term. In these cases, the biographic disruption due to the diagnosis of breast cancer has to consider both the implications on the women's life and the construction of the maternal role. Psycho-decisional support is strongly recommended to help women face decisional dilemmas about how to combine disease treatments and their children needs. For instance, in deciding between breast-conserving surgery and mastectomy, women have to consider that postsurgical radiation therapy is avoided [35] because of the high risk of malformations. Furthermore, breastfeeding is contraindicated in breast cancer patients undergoing chemotherapy, especially because of the cytotoxic agents detected in breast milk [36]. Given that breastfeeding is culturally and socially defined as the best healthy option for newborn nutrition, mothers might experience feelings of guilt and failure [33] increasing the level of distress. However, false beliefs on breastfeeding may be spread among breast cancer patients. Since any breast cancer patient's condition is different even in the possibility of breastfeeding, the patient's beliefs that breastfeeding after the treatment can be dangerous either for the newborn or for the possibility of cancer recurrence have to be considered and discussed with the patient, in order to avoid any possible false beliefs and consequent low-quality decision-making.

In supporting pregnant breast cancer patients, a particular attention has to be dedicated to confidence towards the health staff professionals: in doctor-patient relationship, many ethical frameworks can emerge. Professionals have to care for women and for their children, and opinions may differ about benefits and risks of decisions on medical treatments. Professionals may feel the double responsibility for women's and foetus's lives, facing high distress.

In this sense, psychological support to professionals in emotional management and support to medical decision-making are recommended too. The best option would be providing patients with a personalized approach through the implementation of a multidisciplinary team and the coordination of communication among professionals.

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HIFU and Radio Frequency as Alternatives to Surgery

74

Franco Orsi and Giovanni Mauri

74.1 Introduction

Although the incidence of breast cancer is increasing in the last 10 years, the mortality rate was reduced by 20% due to the earlier tumor diagnosis (more and more frequently non-palpable intraepithelial neoplasia) and to improved therapies, tailored to each single patient [1–3].

Over the last four decades, the breast cancer surgical and nonsurgical treatments have undergone continuous and deep changes thanks to the improvements in diagnostic imaging developments and the diffusion of wider screening programs that have allowed the detection of subclinical small tumors [4].

Regarding the local treatments, the breast-conserving surgery has been progressively validated as a safe and effective alternative to radical mastectomy, for those patients diagnosed with an early-stage breast cancer [5–8]. The first world trial on this new concept began in Italy at the Milan Cancer Institute in 1973. It reported the same long-term survival rate with acceptable rates of local relapse between breast-conserving surgery (quadrantectomy and complete axillary dissection followed by radiation therapy) and radical mastectomy, but with a much better cosmetic result in the first group of patients [5]. Few years later, another large trial in the USA obtained the same result [8].

In the recent years, there has been a further trend to progressive reduction in invasiveness of local treatments, aiming to achieve the same result as standard options, but with less morbidity and a better quality of life that opened up new horizons toward minimally invasive techniques in several different fields of oncology, such as percutaneous treatment of liver and kidney tumors [9–12]. Recently, following experience in other oncologic disciplines where they demonstrated high efficacy in achieving local control in several types of malignancies, some studies focused on application of minimally invasive image-guided ablations in breast cancer care [13–46].

Among the percutaneous minimally invasive thermal ablation techniques, the radio frequency ablation (RFA), the cryoablation, the laser ablation therapy (LA or LITT), and the microwave ablation (MWA) were investigated in early breast cancer local treatment and reported as comparably feasible and safe, but to be still considered at the investigative stage. The rationale behind these thermal ablation techniques is the absolute sensitivity of biological tissue to the heat. Every cell is supposed to die, due to protein denaturation and the subsequently coagulative necrosis, if exposed to a temperature higher than 50 °C for 4–6 min.

In this scenario, the advent of more and more precise and sophisticated imaging tools has led to a resurgence of interest in an old "noninvasive" thermo-ablative technique, based on the use of ultrasound energy. The high-intensity focused ultrasound (HIFU) is a highly precise medical procedure, which employs focused ultrasound energy to burn and destroy the tumor tissue deeply located within the body, selectively, and without harming overlying and adjacent structures within the path of the beam [47, 48].

The idea of developing an ultrasound-focused therapy based on controlled local heating phenomena was introduced by Lynn et al. in the 1940s, but the technique was not developed at that time because of inadequate imaging guidance as targeting methods. The development of the new diagnostic imaging tools, such as ultrasound, magnetic resonance, and computed tomography, has led to a resurgence of interest in HIFU therapy [48, 49].

Unlike RFA/MWA or cryoablation, which are also used to percutaneously ablate tumors, HIFU is completely noninvasive and can be used to reach tumors that are deep within the body. The presence of an adequate acoustic window for allowing for the transmission of ultrasound energy is crucial for the feasibility of this technique. Preliminary reports underlined a reduced toxicity with HIFU ablation compared with other ablation techniques because of the noninvasive nature of the procedure.

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First devices widely used in clinical practice were transrectal probes, which have been used predominantly to treat prostate cancer. Extracorporeal devices are significantly larger and can be used to treat a variety of problems, most commonly intra-abdominal solid tumors. As a result, these extracorporeal devices use transducers with a longer focal length and use both US and MRI for targeting the organ.

74.2 Basic Principle of HIFU

Higher energy and intensity are used in therapeutic purpose than in diagnostic US, though HIFU principles are the same as conventional US. The main mechanisms of HIFU ablation involve mechanical and thermal effects at the level of the "focal point," where US energy is focused and concentrated.

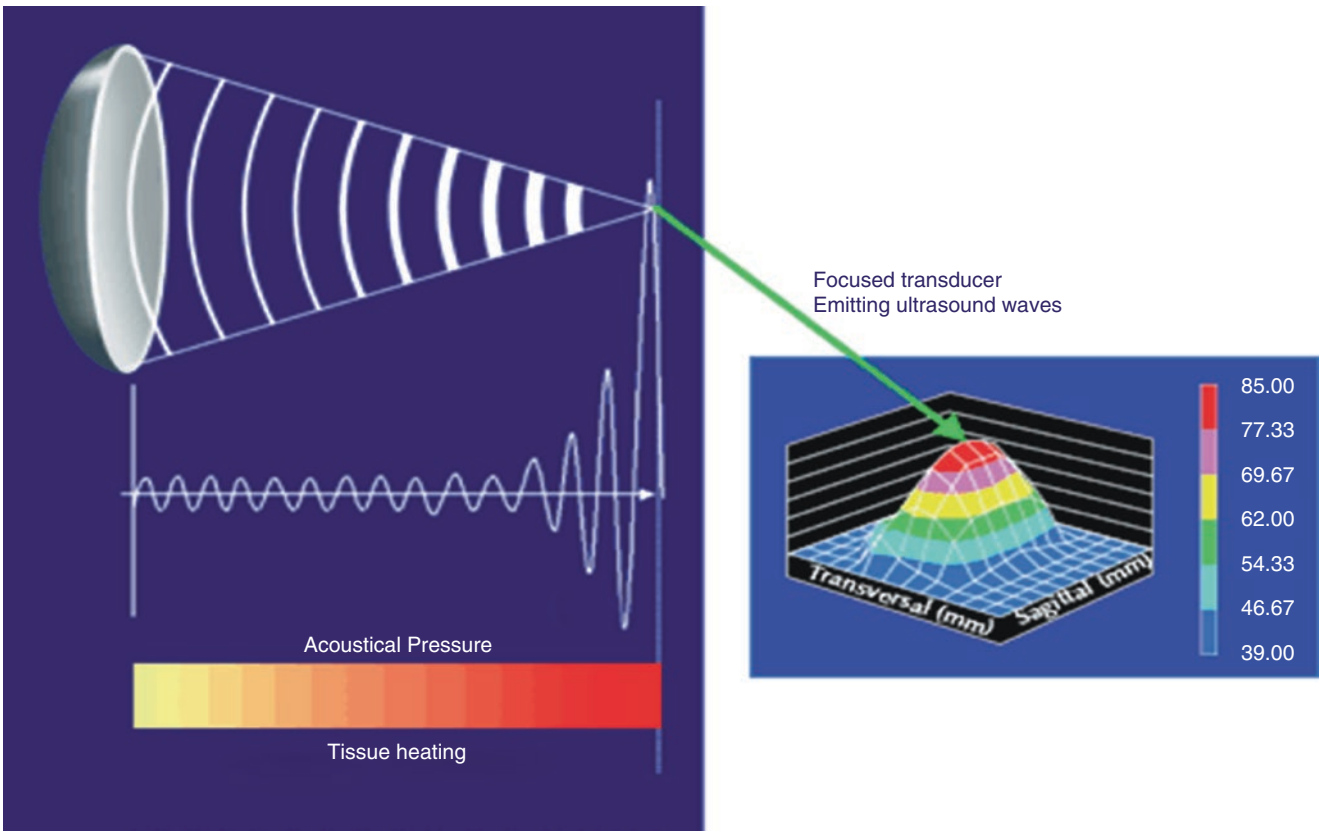
Localized heat generation due to absorption of the acoustic energy is the main biological effect in tissues where it rapidly raises temperatures from 55 to 100 °C, causing coagulation necrosis within a few seconds. The precise and well-delimited US focusing minimizes the potential thermal damage to the tissue located along the acoustic pathway, because the energy is much lower outside the focal region. Mechanical phenomena, in addition to thermal effects, are associated only at high energies and include micro-streaming, radiation force, and cavitation.

Cavitation is the most important mechanical phenomenon; it can be defined as the creation or motion of gas bubbles within an acoustic field due to alternating compression and expansion of tissue as ultrasound waves propagate through it. Thermal effects, acoustic cavitation, and vessel damage can occur simultaneously within the targeted tissue in HIFU ablation. Therefore, the coagulation necrosis induced by HIFU can be considered as the result of biological effects from a combination of heat, cavitation, and vascular destruction on tissue. Combination between imaging and technologies for local therapy has made ablative procedures more reliable and practical, allowing for safe and feasible application of HIFU treatments in clinical practice.

Clinical HIFU procedures are generally combined with magnetic resonance imaging (magnetic resonance -guided focused ultrasound = MRgFUS) or ultrasound imaging (ultrasound-guided high-intensity focused ultrasound = USgHIFU) as image guidance and treatment monitoring [47–49].

Guidance and monitoring of acoustic therapy is a crucial issue to ensure that the desired region is treated and for minimizing the possible damage to the adjacent structures.

Both MRI and ultrasound guiding methods have their advantages and disadvantages. Real-time imaging, such as US, ensures that HIFU beam targeting is maintained within the correct area throughout the procedure. MRI has the



advantage of providing temperature data within seconds after HIFU exposure (sonication). The MRgFUS procedure in clinical setting was established for the treatment of symptomatic benign uterine tumor and palliative treatment for patients with painful bone metastases.

However, MRI guidance is expensive, labor-intensive, and of lower spatial resolution in some cases, although it is superior to sonography in obese patients. Sonographic guidance provides the benefit of using the same form of energy that is used for therapy. The significance of it is that the acoustic window can be verified according to the US findings. In addition, the use of contrast-enhanced ultrasound (CEUS) can be also used for evaluating efficacy of HIFU treatment immediately after treatment.

74.3 HIFU in Breast Cancer

In the present era of conservative medicine, few studies on limited number of patients investigated the application of different minimally invasive techniques in the treatment of breast cancer [50–59]. With all the classical methods of percutaneous thermal ablation, including RFA, cryoablation, MWA, and LA, no banal complications as skin burn ulceration and necrosis, often at the puncture site; pneumothorax; moderate pain; and muscle burn were recorded.

The main reason of the very small diffusion of these methods is probably the percutaneous approach and the reported side effect if compared to the well-established surgical technique.

Today, among all the minimally invasive techniques, high-intensity focused ultrasound ablation is the only really noninvasive option available that doesn't cause any direct skin damage due to the needle insertion and doesn't use ionizing energies.

In 2001, Huber et al. described the first MRgFUS treatment in a breast cancer patient [50]. Subsequently, other authors reported their experience on MRgFUS ablation of malignant breast tumors prior to surgical resection, with the common statement that MRgFUS ablation is technically feasible in breast cancer. Complete tumor necrosis, however, was achieved in only 20–50% of patients [51–54].

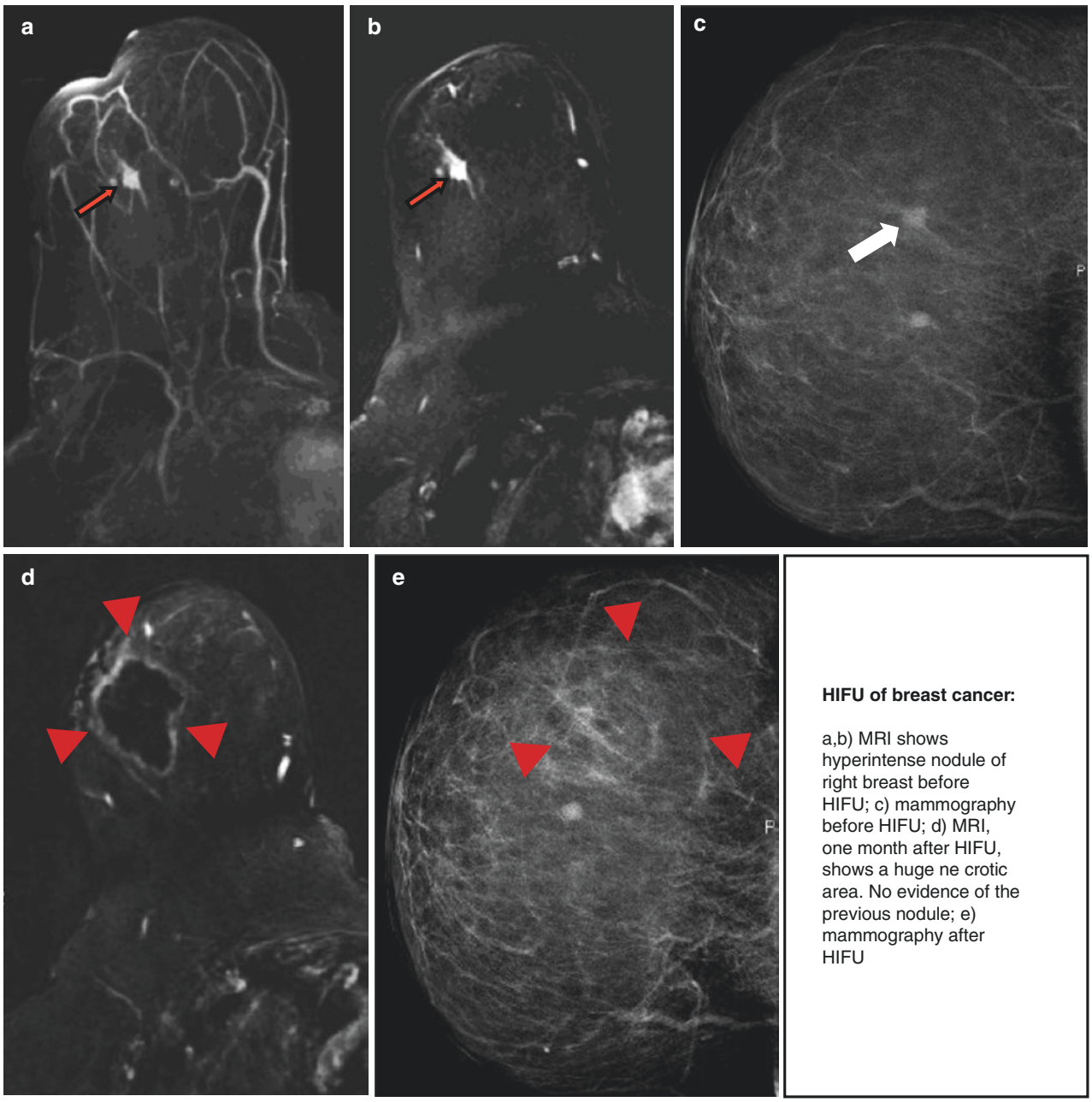
Wu et al. showed complete necrosis of tumor cells by HIFU technique and concluded that HIFU system could be safe and effective in localized breast cancer treatment, but more clinical trials are needed for defining the future role of this promising technique [55]. In Li's review on breast cancer treatments using HIFU, guided by US or MRI, between 2002 and 2010, 173 patients with tumor extent from 0.5 to 6.0 cm were reported. Complete ablation rate was 71% (123/173) by MRI-guided HIFU or US-guided HIFU therapy [58]. The complete necrosis rates were 59% (71/121) by MRI-guided HIFU and 96% (50/52) by US-guided HIFU therapy.

There are several advantages of HIFU therapy for breast cancer treatment, including preserving the structure and function of breast, no bleeding, no scarring, and no radiation. Li A and Wu PH [58] reported three major limitations related to HIFU ablation: (1) it is difficult to confirm whether ablation margin is free, (2) recurrence concern exists in multifocal breast cancer, and (3) necrotic masses remaining in the breast after HIFU therapy could cause additional psychological burden to the patient.

A recent systematic review of HIFU ablation for breast cancer treatment showed a lack of reports for consistent histopathology, addressing for large, prospective clinical trials evaluating results according to lesion histopathology and imaging follow-up for margin necrosis assessment [59]. Because of the need of these crucial histopathological feedbacks after HIFU, between December 2007 and April 2010, an open prospective, single-center nonrandomized phase 2 study on USgHIFU for breast cancer was conducted at the European Institute of Oncology. Healthy adult woman affected by unifocal early breast cancer visible at US imaging was considered eligible for this "ablate and resect" study. Eighteen patients underwent USgHIFU ablation for 18 previously core-biopsied tumors up to 15 mm, before undergoing surgery for histopathological assessment. The median value of tissue necrosis percentage obtained, evaluated by HE staining, was 95%. In seven patients, a complete necrosis (100%) was showed, and in two patients it was more than 95%. However the TTC evaluation for biological status showed a complete necrosis in 16 patients. In three cases with complete absence of tumor, the lesion was vanished by cells' cavitation and vaporization. In one patient, the lesion appeared perfectly intact with no necrosis, because it was completely missed during HIFU treatment.

In our preliminary experience, based on this "pilot study," among the other local treatments for breast cancer, HIFU has the advantages to be completely noninvasive and feasible under sedation and local anesthesia, in a day hospital setting, allowing for considerable cost reduction compared to traditional surgery. The risks of surgery-related complications (e.g., infection, seroma, and bleeding) are also eliminated.

In conclusion, USgHIFU treatment was shown to be reliable and feasible in unifocal early breast cancer. We can be very confident in this method that, with necessary improved technology, will take the lead among the alternative treatments to surgery, but several larger and really well-conducted



HIFU of breast cancer:

a,b) MRI shows hyperintense nodule of right breast before HIFU; c) mammography before HIFU; d) MRI, one month after HIFU, shows a huge ne crotic area. No evidence of the previous nodule; e) mammography after HIFU

clinical trials are needed before considering this noninvasive approach as an effective cancer treatment in breast disease.

74.4 Radio Frequency Ablation in Breast Cancer

Radio frequency (RF) ablation is the first technique that was used to perform image-guided thermal tumor ablation. Traditionally used in the liver, RF is applied through an electrode (i.e., the needle) under imaging guidance to induce focal high-temperature cytotoxic heating in target tumors. The patient is part of a closed-loop circuit made by the needle, the power generator, and a ground pad. This allows for having an alternated electric field within the tissue of the patient. As biological tissues are poor electricity conductors, ionic friction takes place and leads to heat generation (i.e., the Joule effect). The discrepancy between surface of the needle and of the ground pad allows for concentrating most heating power around the needle itself. Thus, up to 5 cm of tissues around the needle tip can be focally heated up to 100 °C. High temperature implies tissue dehydration

and water vaporization, thus leading to coagulative necrosis. Different technical solutions (e.g., needle shape and length, cooling systems, etc.) allowed for improving the performance of RF in various body districts. One drawback of RF system is that margins of ablation volume can be poorly predicted. The first experience with RF ablation of breast cancer is dated 1999, and five patients were treated with complete ablation in four of them [13].

The evidence regarding these techniques is quite sparse, especially if we consider not only the technical success but also the efficacy and the rate of complications. Moreover, published studies frequently enrolled small groups of patients not allowing for drawing reliable conclusions.

From a recent systematic review and meta-analysis on 576 patients treated with RF ablation for breast cancer, technical success of the technique has been found to range between 87% and 100%, with a largely variable technical efficacy, ranging between 29% and 100%. The large variability of technical effectiveness in comparison with the elevated rate of technical success might be ascribed to various factors, including the small number of cases treated in each series (reflecting an initial experience with the technique), the wide difference in treated tumor sizes, and the different histological subtypes.

In the analyzed papers, major complications were reported to occur in a range between 0% and 8% and minor complications in the range between 4% and 62%, being the most frequent complications represented by skin burns grade 2 or 3, tissue necrosis, and pneumothorax.

In conclusion, RF ablation of breast cancer has been reported as a feasible technique, with low rate of serious complications. However, results in terms of efficacy are still quite inhomogeneous, and several factors, which might affect the result of the procedure, have not been investigated yet. Thus, even if image-guided percutaneous ablations of breast cancers, and in particular RF, seem to offer some advantages over surgery and promising results, the available clinical experiences are still too limited to propose their application in the clinical practice. Further studies are needed to better clarify the best technology for performing percutaneous image-guided ablations of breast cancer and the correct indication for a wider clinical application.

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Hyperthermia with Radiotherapy and with Systemic Therapies

75

J. van der Zee and G.C. van Rhoon

75.1 Introduction

Hyperthermia (HT) is a treatment during which the temperature of tissues is increased a few degrees above the physiological level, to a range of 39–45 °C. The modern era of this treatment modality started in 1975, when the first international hyperthermia meeting was held in Washington DC. The clinical experience presented at that meeting was limited to the application of fever therapy, whole-body HT and perfusion of limbs or the bladder. From experimental studies it had become clear that HT would be useful mainly in combination with radiotherapy (RT) or chemotherapy. The first available techniques for clinical application of hyperthermia with ultrasound or electromagnetic radiation had been developed. After this meeting, many institutes started clinical research on hyperthermia. Most clinical research groups started with treating patients who were progressive after all kinds of other therapies. Nowadays, a number of randomized trials have demonstrated that HT, in addition to radiotherapy (RT) and/or chemotherapy, considerably improves the clinical outcome in patients with a large variety of tumours. Among the first treated patients were patients with breast cancer recurring after previous RT, in whom HT was combined with relatively low doses of reirradiation (reRT). Presently, recurrent breast cancer after previous RT is one of the indications where HT is considered part of standard treatment.

75.2 Rationale for Hyperthermia in Cancer Treatment

Hyperthermia at a temperature of 39–45 °C has various effects. One effect is direct cell kill, for which the main molecular event is protein damage [1]. Cell death may result directly or from triggering apoptotic pathways [2]. The effect depends on the height of temperature and the duration of treatment, which two factors determine HT dose. Environmental factors such as hypoxia and low pH, which under normal conditions are present only in tumour tissue, due to insufficient perfusion, make cells more sensitive to an increased temperature. When both a tumour and its surrounding normal tissues are heated to a similar high temperature of up to 43 °C, part of the tumour cells will be killed without damage in the normal tissues. In patients with breast cancer, it was demonstrated that the HT dose needed for cell necrosis and vessel damage is higher in normal tissue than in tumour tissue [3]. The effects of HT specifically in regions of hypoxia and low pH make the treatment complementary to both RT, for which cells in a hypoxic environment are less sensitive, and chemotherapy, since the drug concentration will be lower in a region with insufficient perfusion.

Besides inducing direct cell kill, HT sensitizes cells to both RT and a number of cytotoxic drugs. One important mechanism for this sensitizing effect is inhibition of DNA repair [4, 5]. The magnitude of this sensitizing effect of hyperthermia is called thermal enhancement ratio (TER): the ratio between the RT and chemotherapy dose needed for a certain effect, divided by the RT or chemotherapy dose required for the same effect when combined with HT. Hyperthermia may further increase the effects of RT and chemotherapy by an increase in blood flow [6]. An increased blood flow may result in an increased oxygenation, which makes radiotherapy more effective. When combined with chemotherapy, an increased blood flow will increase the drug delivery to the heated tissues.

75.2.1 Radiosensitization

Heat is probably the most potent radiosensitizer known [7]. The sensitizing effect of HT depends on height of temperature and duration of treatment and decreases with longer time intervals between the two treatment modalities. For example, with

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HT and RT applied simultaneously, the TER was 5 for 90 min heating at 43 °C in a mouse mammary carcinoma. When HT was applied 4 h after RT, the TER was 2 for the same HT dose. In clinical comparative studies, iso-effect TERs of 1.5–1.6 have been found [8]. Experimental studies have shown that the radiosensitizing effects disappear faster in normal tissues than in tumour tissues, so that the therapeutic ratio is maximum when hyperthermia follows radiotherapy after 2–4 h [9]. For the radiosensitizing effect of HT, nuclear protein damage is probably the key event [1]. The heat-induced nuclear protein aggregation leads to inhibition of repair of radiation-induced DNA damage, resulting in more lethal DNA lesions.

75.2.2 Chemosensitization

The effects of several drugs are enhanced by HT [10]. The TER depends, also for chemosensitization, on the height of temperature and the duration of treatment. For various drugs, TERs of 1.05–3.6 were found at 41.5 °C and of 1.6–2.7 at 43.5 °C. The mechanisms for sensitization are an increased rate of alkylation, an increased drug uptake and inhibition of repair of drug-induced sublethal damage. Some drugs are only enhanced above a threshold temperature, e.g. bleomycin, doxorubicin and actinomycin are sensitized only at temperatures above 42 °C, while platinum drugs and alkylating agents show a gradual increase of effect with increasing temperatures. The optimal sequence is simultaneous application for most drugs, provided that the organ with dose-limiting toxicity is not included in the heated volume. For gemcitabine, synergism was only observed when it was applied 24 h before or after HT.

An interesting approach is to combine HT with thermosensitive liposomes. Hyperthermia may increase the local blood flow and further increases the gaps between vascular endothelial cells. As a result, more liposomes will enter the interstitial space and release their contents within the heated tissue. Experimental studies have shown that more drugs enter the tumour by this approach and also that the therapeutic effect is increased [11].

75.2.3 Immune Modulation

Hyperthermia modulates innate and adaptive immune responses. When a tumour cell is heated, protein aggregation and denaturation induces a stress response in the cell, resulting in an increase in the transcription of inducible heat shock protein 70 (HSP70) and an increase in exposure of HSP70 on the cell surface. HSP-expressing cells are more susceptible to lysis by natural killer effector cells. Furthermore, hyperthermia results in enhanced levels of tumour antigens inside the cell. If the treatment results in cell death, HSPs acting as danger signals and HSP/antigen complexes are released. In addition, HSPs and tumour antigen-containing exosomes can be discharged from apoptotic and necrotic tumour cells. Such exosomes as well as the Hsp/antigen complexes activate and attract dendritic cells. These take up tumour antigen, present it with costimulation to CD8 + T cells and thereby induce cellular antitumour immunity by priming cytotoxic T lymphocytes which then attack and may kill the tumour cells [12]. Tumour growth in a rat model was significantly inhibited following a pre-implantation heat treatment, while splenic lymphocytes displayed specific cytotoxicity against the implanted cells [13]. The remarkable clinical outcome in the patient presented in Fig. 75.1 and the reported overall survival of 66–75% after 3 years and 60% after 5 years in patients treated with reirradiation and hyperthermia after resection of recurrent tumour (see below) may be the result of such an immune stimulation.

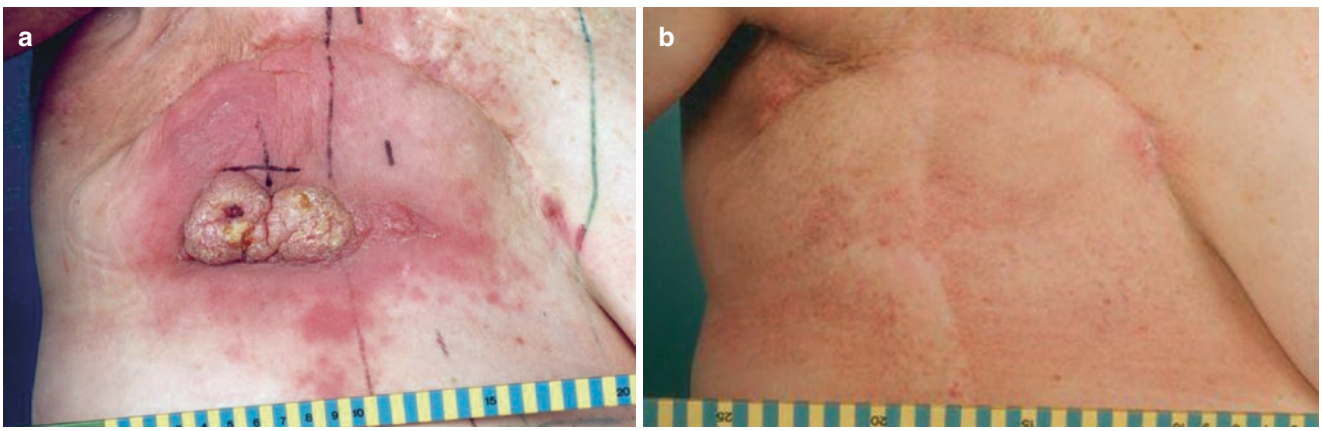

Fig. 75.1 This patient was primarily treated with lumpectomy and radiotherapy. Within 2 years, mastectomy followed after tumour recurrence. Six months later, she had a large and fast-growing tumour on the chest wall (**a**), which was treated with reirradiation (eight fractions of 4 Gy twice weekly) and hyperthermia, resulting in a complete response (**b**). Up to the last follow-up, 16 years after reirradiation and hyperthermia, there was no evidence of disease

75.3 Techniques for the Application of Hyperthermia

Hyperthermia can be applied to the whole body, regionally or locally. Breast cancer is mainly treated with local HT. Local HT can be applied by external or interstitial methods. Energy is delivered to the tissue with ultrasound or electromagnetic radiation. The volume that can be heated depends on the physical characteristics of the energy source and on the type of applicator. The energy distribution in the tissues is strongly dependent on tissue characteristics and thereby inhomogeneous. The resulting temperature is not directly related to the local energy absorption, but also depends on thermal characteristics of the tissue, the blood flow and the temperature of the surrounding [14]. For interstitial HT, small applicators are inserted into the tissue, usually within catheters that are used for brachytherapy as well. A disadvantage of interstitial HT is that the temperature decrease in the tissue around the applicator is very steep. Most clinical experience in breast cancer patients is with external HT by (non-ionizing) electromagnetic radiation. The depth to which tissues can be heated adequately decreases with increasing frequencies and can be influenced by a perfused water bolus on the skin surface [15]. Hyperthermia treatment planning is available, which helps to select the optimum heating technique for specific patients [16]. For the treatment of recurrent breast cancer, which usually requires heating of a large area, multi-applicator systems have been developed (like the one presented in Fig. 75.2). Temperatures should be preferably measured both interstitially and on the surface, so that the power output of the applicators and the temperature of the water bolus can be adjusted for the achievement of an optimized temperature distribution.

75.4 Clinical Results

75.4.1 Clinical Results in General

In most clinical studies, HT is applied once or twice weekly during one hour. Fourteen early studies with HT as single modality have shown clinical complete response (CR) rates of 0–40%. Overall, HT alone to total 433 lesions resulted in 14% clinical CR [17]. These lesions usually were small and superficially located and could be heated well. The CRs after HT alone generally were short-lasting; it is therefore not recommended to use HT as single modality. Several studies have compared the effect of RT to that of RT plus HT in patients with multiple lesions. In some of these studies, the treatment was randomly selected; in other studies the larger lesions were treated with the combination. In all these studies, the CR rate was higher after combined treatment than after RT alone. A summation of the data of all these studies, with total 713 lesions, shows CR rates of 31% and 67% after RT alone and RT plus HT, respectively [17]. A number of randomized trials have given evidence of considerable improvement of clinical outcome from addition of HT to RT, chemotherapy or both. Benefit has been shown in a large variety of tumour types, including breast cancer, and for various outcomes, including local tumour control (LC) and overall survival [17].

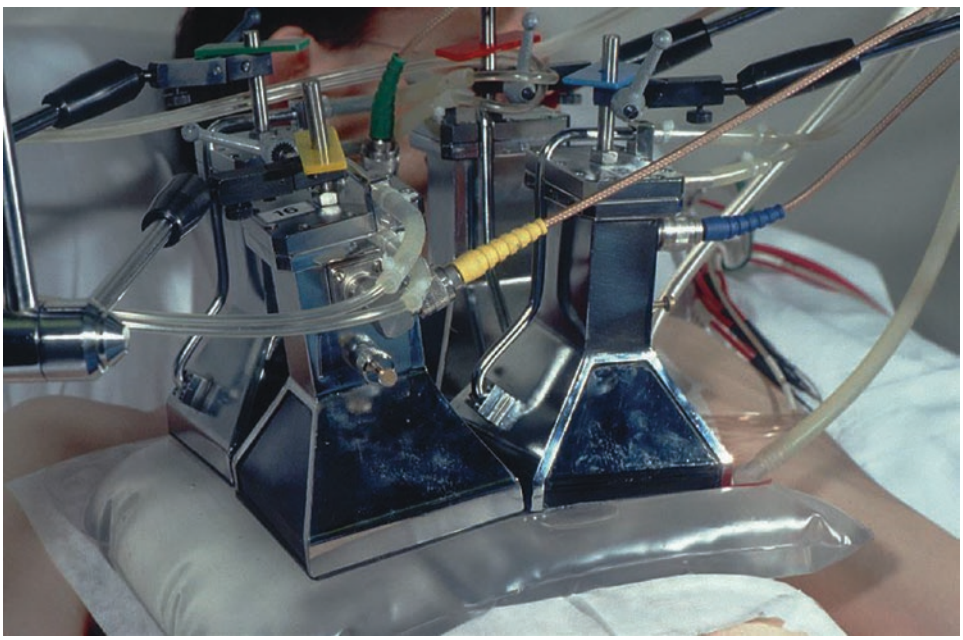

Fig. 75.2 Applicator set-up for treatment of the thoracic wall (Rotterdam). This custom-built system uses 433 MHz electromagnetic radiation for heating. Up to six applicators can be used simultaneously, to treat an area of 20 × 30 cm^2 to a depth of up to 4 cm

From clinical results of HT combined with RT for locally advanced or recurrent breast cancer, a TER of 1.5 was derived [8]. One study combining five separate randomized trials comparing RT to RT + HT included patients with primary breast cancer, recurrent breast cancer and recurrent breast cancer after previous radiation [18]. The difference in CR rate between the radiotherapy alone (41%) and the combined treatment arm (59%) was significant. After 3 years of follow-up, the local control rate in the RT-alone arm was 24% and in the combined treatment arm 46% ($p = 0.007$). In this study the strongest effects of HT were seen for patients treated with reirradiation: the CR rate increased from 31% after reRT alone to 57% after combined treatment.

75.4.2 Toxicity

Hyperthermia may damage normal tissues when the tolerance limits are exceeded. Most tissues tolerate treatment of 1 h at 43 °C; only nervous and gastrointestinal tissues appear more sensitive [19–21]. Tissues with disturbed blood flow will heat easier and, when oxygen supply is decreased, will be damaged at lower temperatures. During treatment, it is therefore important to avoid pressure on normal tissues. It is not always possible to avoid high temperatures in normal tissues, due to the heterogeneity of temperature distribution and the limited thermometry. Patients are instructed to mention unpleasant feelings which may result from a hot spot, but this is not always possible, e.g. after surgery in the past resulting in disturbed sensitivity. Toxicity of superficial HT is most often a skin burn, in 9–28% of patients with recurrent breast cancer [22–28], which heals with conservative treatment. In the randomized studies comparing between RT and RT plus HT, no significant differences in acute or late radiation toxicity were found [17]. In the studies combining hyperthermia with chemotherapy, toxicity was not higher than was expected from chemotherapy alone.

75.4.3 Hyperthermia in Primary Breast Cancer

Hyperthermia has been applied to primary breast cancer by several groups. Only two studies were comparative. Savchenko et al. [29] published in 1987 results of a randomized study in 507 patients with advanced breast cancer, preoperatively treated with RT, with or without HT. The patients treated with the combination tended to have a better survival, 87% and 73% after 3 and 5 years, while it was 77% and 67% for patients treated with RT alone. In the study by Vernon et al. [18], results in primary breast cancer (total 30 patients) were not significantly different between the two treatment arms.

Jones et al. [26] treated 18 patients with advanced breast cancer preoperatively with three cycles of taxol, RT to a total dose of 50 Gy and HT twice weekly. The clinical response rate was 83% including six (33%) complete responses, which was considered high. Of the 13 patients who underwent mastectomy, 3 patients showed a pathological CR. Vujaskovic et al. [27] treated 47 patients with preoperative liposomal doxorubicin, paclitaxel and hyperthermia. This treatment was well tolerated. Eight patients had breast conservative treatment thereafter; 39 underwent modified radical mastectomy. There were 4 pathological complete responses (9%) and 22 partial responses (51%). They found more pathological responses with higher HT doses, which is important since a pathological response is related to a favourable overall outcome. Hofman et al. [28] treated 40 patients with advanced tumours, mainly T3 and T4, with RT (50 Gy) and HT. Clinically a complete response was achieved in 39%. They concluded that for better results, the RT dose should be increased and the quality of the HT treatment improved.

In two studies, interstitial HT was applied in combination with brachytherapy. Chichel et al. [30] treated 57 patients with brachytherapy after lumpectomy. In 32 patients they applied interstitial HT as well. After median 40 months of follow-up, the LC rate was 100% for both treatment groups. Hartmann et al. [31] combined preoperative chemotherapy (several types) with RT and HT in 158 patients with stage IIA–IV breast cancer. Radiotherapy consisted of an interstitial RT boost of 10 Gy followed by interstitial HT at 43.5–44.5 °C for one hour. Thereafter, external beam RT was given to a total dose of 50 Gy. Breast conservative surgery was possible in 52% of the patients. After a median follow-up period of 20 months, a local recurrence was observed in only 1 patient (0.6%). These results were considered promising, since more than half of the patients had a tumour diameter of more than 5 cm.

75.4.4 Hyperthermia and Radiotherapy in Recurrent Breast Cancer

Published results of primary RT combined with HT are scarce. The largest series is reported by Refaat et al. [32]. Eighty-five patients were treated with 50–60 Gy. The CR rate was 61% and after median 13 months LC rate was 55%. Amichetti et al. [33] report 100% CR in eight patients treated with 60 Gy and HT and a 3-year LC rate of 88%. Scott et al. [34] compared the CR rate following RT alone to that following RT plus HT in 17 patients with multiple lesions. The total radiation dose was 60–66 Gy for all lesions. Complete response was achieved in 47% of the lesions treated with RT alone and in 94% of the lesions treated with the combination.

A similar comparative trial is reported by Li et al. [35]. Ten patients with primary breast cancer and 30 patients with recurrent breast cancer were treated on total 64 lesions. The average total RT dose was 47 Gy. For the larger lesions (average 38.7 cm^2), RT was combined with HT; RT alone was applied to smaller lesions (average size 12.1 cm^2). Complete response rates were 36% after RT alone and 64% after combined treatment.

75.4.5 Hyperthermia and Reirradiation in Recurrent Breast Cancer

Patients with an irresectable recurrent tumour after previous radiotherapy to the same area have limited treatment options. Reirradiation (reRT) is the only local treatment option, but the dose that can be given without a high risk of unacceptable toxicity is lower than considered adequate [36, 37]. Furthermore, tumours growing in irradiated tissues are less sensitive to RT and systemic therapy [38]. An uncontrolled recurrent breast cancer will cause problems such as pain, bleeding, ulceration with bad smell and the psychological burden of seeing a growing tumour, while the patient still may have considerable time to live. Many of the centres starting with applying local HT used it for treating patients with this condition, in combination with low doses of reirradiation. An early publication by Tait et al. [39] describes a study in patients with multiple lesions. Lesions were treated with various doses of RT, which in some lesions was combined with HT. From the differences in growth delay, the TER was calculated. For patients with breast cancer, the TER varied from 1.1 to 3.4, with a median of 2.2.

The study by Vernon et al. [18] clearly demonstrated a benefit from adding HT to reRT, with the CR rate increasing from 31% following reRT alone to 57% following reRT and HT. Van der Zee et al. published in 2010 [40] an overview of all studies on reRT and HT in patients with previously irradiated breast cancer, published between 1981 and 2008. The CR rate varied from 28% to 58% (overall 32% in 170 patients) after reRT, while it was 20% to 95% (overall 61% in 974 patients) after combined treatment. Since then, several more studies were published, which are summarized, together with studies in which reRT alone was applied, in Table 75.1. Two studies using RT alone at mean doses of 47 and 48 Gy report CR rates of 36% and 39%, respectively, which is lower than the CR rates of 52–70% reported for lower doses of RT combined with HT. Harms et al. treated 30 patients with RT using a brachytherapy mould, with two fractions of 20 Gy. They report a CR rate of 80% and 3-year LC of 75%.

Table 75.1 Results after reirradiation, with or without hyperthermia, for irresectable breast cancer recurrences

Reference	RT dose	CR after RT (total *n*)	CR after RT + HT (total *n*)	3-year LC	5-year LC
With HT					
[40] Review of 24 studies	Predominantly 30–40 Gy	28–58% (170) 32%	20–95% (974) 61%		
[41]	Mean 40 Gy		(241) 52%		
[25]	32 Gy		(248) 70%	40%	39%
[42]	Mean 22.5 Gy		(72) 69%		
[24]	32–36 Gy		(414) 58%	25%	
Without HT					
[43]	Mean 47 Gy	(22) 36%			
[44]	2 × 20 Gy	(30) 80%		75%	
[45]	Mean 48 Gy	(37) 39%			

RT radiotherapy, *HT* hyperthermia, *CR* complete response, *LC* local tumour control

75.4.6 Hyperthermia and Reirradiation in Recurrent Breast Cancer, After Resection

Patients with resectable recurrent breast cancer have a high risk of recurrence when the resection margin is tumour positive (R1 resection) and, in case of close margins, a large or multicentric tumour or multiple recurrences on the same location in the past. With radiotherapy doses that are considered appropriate for elective treatment (45–50 Gy) or for treatment of microscopic disease (50–60 Gy) [36, 37], the risk of unacceptable toxicity is too high. Four publications report on local control rates after postoperative reRT and HT of breast cancer recurrences. Kapp et al. [46] evaluated local control in 262 fields treated electively in 89 patients, of which 54% had been treated with RT before. The mean RT dose was 42 Gy. LC rate at 3 years was 74%. Two studies evaluated LC after reRT with eight fractions of 4 Gy, twice weekly, combined with once weekly HT, after resection of recurrent disease. Oldenborg et al. [22] treated 78 patients of whom 39% after resection with tumour negative margins (R0 resection) and 61% after R1 resection. LC rates after 3 and 5 years were 78% and 65%, respectively. In this study, 3-year overall survival after reirradiation was 66%. Linthorst et al. [25] treated 198 patients of whom 54% after R0 and 46% after R1 resection. LC rates were 83% and 78% after 3 and 5 years, respectively. Three-year and 5-year overall survival

Table 75.2 Results after (re)irradiation of recurrent breast cancer, with or without hyperthermia, after tumour resection

Reference	RT dose	2-year LC	3-year LC	5 -year LC
Reirradiation with HT				
[41] 195 fields	Mean 44 Gy	74%		
[23] 198 pts	32 Gy		83%	78%
[22] 78 pts	32 Gy		78%	65%
[47] 14 pts	Median 60		86%	
Reirradiation without HT				
[44] 28 pts	2 × 20 Gy		71%	
[47] 2 pts	Median 60		50%	
Primary radiotherapy, without HT				
[48] No adjuvant tamoxifen	50 Gy		77%	71%
Adjuvant tamoxifen			90%	87%
[49]	50–60 Gy		60%	
[50]	60 Gy			48%

RT radiotherapy, *HT* hyperthermia, *LC* local tumour control

after reirradiation were 75% and 60%, respectively. In spite of the relatively low reRT doses, the LC rates after reRT plus HT are comparable to the results after higher RT doses which have been applied, without HT, postoperatively for primary or recurrent breast cancer (summarized in Table 75.2).

75.4.7 Hyperthermia and Chemotherapy in Recurrent Breast Cancer

Zoul et al. [51] combined paclitaxel with local hyperthermia in a pilot study on seven patients. Patients had an inoperable local recurrence after mastectomy, irradiation and systemic therapy. Paclitaxel infusion was followed by 45 min of hyperthermia, for 6–18 cycles. All patients responded: complete response in four and partial response in three. There were no toxicities higher than grade 2. Three of the complete responders were still locally controlled after 12, 13 and 16 months, which seems a rather good effect.

75.4.8 Combination of Hyperthermia with Radiotherapy and Chemotherapy in Recurrent Breast Cancer

Feyerabend et al. [52] combined RT with epirubicin and ifosfamide, in which drugs were given once weekly, during HT. In patients who were reirradiated (reRT dose 36–60 Gy), a CR was achieved in 4 of 18 patients (22%). In seven patients not previously irradiated (RT dose 50–70 Gy), the CR rate was 86%. Remarkably, tumour progression was observed within 10 months after treatment in eight of the ten complete responders. Zagar et al. [53] treated 27 pts of whom 23 had received radiotherapy in the past with (re)RT to a total dose of median 45 Gy with HT and chemotherapy (capecitabine in 21, vinorelbine or paclitaxel in 6). In 16 of 20 evaluable patients, a CR was achieved (CR rate between 59% and 85%). Kouloulias et al. [54] treated 15 patients with a recurrence after previous surgery and RT with six courses of liposomal doxorubicin combined with HT, of which the first course was given during reirradation with 30.6 Gy. They achieved a complete response in three patients (20%). So far, these results have shown feasibility, but do not indicate a benefit from adding chemotherapy to (re)RT and HT.

Conclusions

The results achieved with hyperthermia added to radiotherapy and/or chemotherapy for primary breast cancer suggest that hyperthermia has the potential of further improving the clinical outcome by adding it to preoperative treatments.

The results of the studies comparing the effect of radiotherapy plus hyperthermia with that of radiotherapy alone in patients with recurrent breast cancer indicate that hyperthermia increases the effect of radiotherapy. Whether the improvement by hyperthermia in addition to full dose radiotherapy is worthwhile still has to be established by a randomized trial.

A beneficial effect of hyperthermia in addition to reirradiation has been established for patients with irresectable breast cancer recurrences. The only study in which radiotherapy alone resulted in a higher complete response rate applied two fractions of 20 Gy with a brachytherapy mould, which is a feasible approach in only a subgroup of patients.

The local tumour control rates after combined reirradiation at relatively low doses and hyperthermia, after resection of breast cancer recurrences, are similar to the local control rates reported for higher doses of radiotherapy after resection of not previously irradiated recurrent tumours. This strongly suggests a beneficial effect of hyperthermia in this patient group as well, although this can only be confirmed by a randomized trial.

The one small study combining hyperthermia with paclitaxel shows that this treatment is feasible and seems effective. The studies performed so far with the combination of hyperthermia with radiotherapy and chemotherapy do not suggest an advantage of the addition of cytotoxic drugs.

In view of the benefits shown for subgroups of patients and the potential benefits in other subgroups of patients, hyperthermia should be available for breast cancer patients, to be used either as part of standard treatment or as an additional modality in clinical studies.

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Electrochemotherapy of Breast Cancer

76

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76.1 Electrochemotherapy: Basic Concepts

Electrochemotherapy is being recognized an effective local ablative therapy that combines administration of a cytotoxic drug with tumor electroporation. At present, established drugs used during electrochemotherapy treatment are bleomycin and cisplatin [1, 2]. Electroporation is a phenomenon induced on cell membrane, which occurs when cells are exposed to appropriate external electric fields. Exposure of cells to high-amplitude electric pulses induces destabilization of the cell membrane (i.e., opening of aqueous pores), which enables diffusion of water-soluble drugs into the cytosol. When specific electrical parameters are selected, the aqueous pores on the cell membrane are transient, and electroporation results in an effective drug delivery system [3].

Bleomycin and cisplatin have high cytotoxicity, but low transmembrane permeability, unless combined with electroporation. In these circumstances, bleomycin cytotoxicity is increased several hundredfold and cisplatin several tenfold according to several observations in animal tumor models. Extensive preclinical data on experimental tumors confirmed the effectiveness of electrochemotherapy on a variety of solid tumors [4].

Several mechanisms of action have been identified in electrochemotherapy. The predominant mechanism is represented by the effective delivery of drug molecules into tumor cells, which is the main effect of electrochemotherapy [1, 5]. The indirect effects of electrochemotherapy are mediated by its vascular-disrupting action. Vascular-disrupting action is caused by a cytotoxic effect of bleomycin or cisplatin on endothelial cells [6]. Noteworthy, recent experimental data indicate that the vascular-disrupting effect of electrochemotherapy occurs selectively on tumor vessels [7, 8]. Specifically, normal vessels in the treated area are spared, while the cytotoxic effect is limited to the tumor vasculature [9]. According to the intriguing results of experiments in animal models, the second indirect mechanism of action observed after electrochemotherapy is the elicitation of a local immune response, which is likely the consequence of tumor necrosis and immunologic cell death [10]. In these studies, complete tumor eradication could be obtained only in immunocompetent mice [11]. It is hope of researchers that electrochemotherapy-induced local immune response may drive a systemic antitumor effect and ultimately lead to complete tumor eradication. With the recent availability of new, highly effective immunomodulatory agents, local tumor response to electrochemotherapy could be switched into a systemic immune response [12–14].

76.2 Electrochemotherapy Procedure

Efficacy of electrochemotherapy relies on two components that needs to be fulfilled. The first is that chemotherapeutic drug must be present in the target tumor tissue in sufficient concentration; the second is the adequate coverage of tumor with electric fields to induce electroporation of tumor cells. Therefore, the procedure consists of drug administration followed by local application of electric pulses. These voltages are generated by an electric pulse generator and are delivered

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by means of different kinds of electrodes that are selected by the clinician according to the specific clinical situations (e.g., tumor size, anatomical location, etc.). Treatment details have been standardized in 2006 thanks to the joint experience of four leading centers performing the procedure and are now available as the European Standard Operating Procedures of Electrochemotherapy (ESOPE) [15, 16].

76.2.1 Drugs and Their Administration

Bleomycin and cisplatin represent the two chemotherapeutic agents used for treatment with electrochemotherapy. Bleomycin is nowadays the drug of primary choice, since it can be administered either intravenously or intratumorally (Fig. 76.1a). According to the ESOPE, instead, cisplatin can be only injected intratumorally [16]. Intravenous bleomycin (at a dosage of 15 units/m^2) is administered in bolus, and the application of electric pulses can be performed starting after 8 min from drug infusion, in order to achieve adequate drug distribution in tumor tissue. The therapeutic window for the application of the electric pulses has been set to 20 min. Nevertheless, recent pharmacological data on elderly (>65 years) patients suggest that it can be effectively extended 40 min or even longer (unpublished data). Intravenous bleomycin is generally used when dealing with patients presenting with multiple metastases, when tumors are larger than 3 cm, and when target tissue has hard consistency, making it difficult to be directly infiltrated. In general, it is a common experience that electrochemotherapy can be safely performed also in elderly patients [17]; however, when repeatedly using intravenous bleomycin, care should be taken not to exceed its recommended maximal cumulative dose (maximum total lifetime dose should not exceed 400 units). On the other hand, intratumoral drug injection, either bleomycin or cisplatin, is indicated for the treatment of patients with a single or few, small-size tumor nodules. In this case, the application of electric pulses to the tumors can be started shortly after drug injection [16].

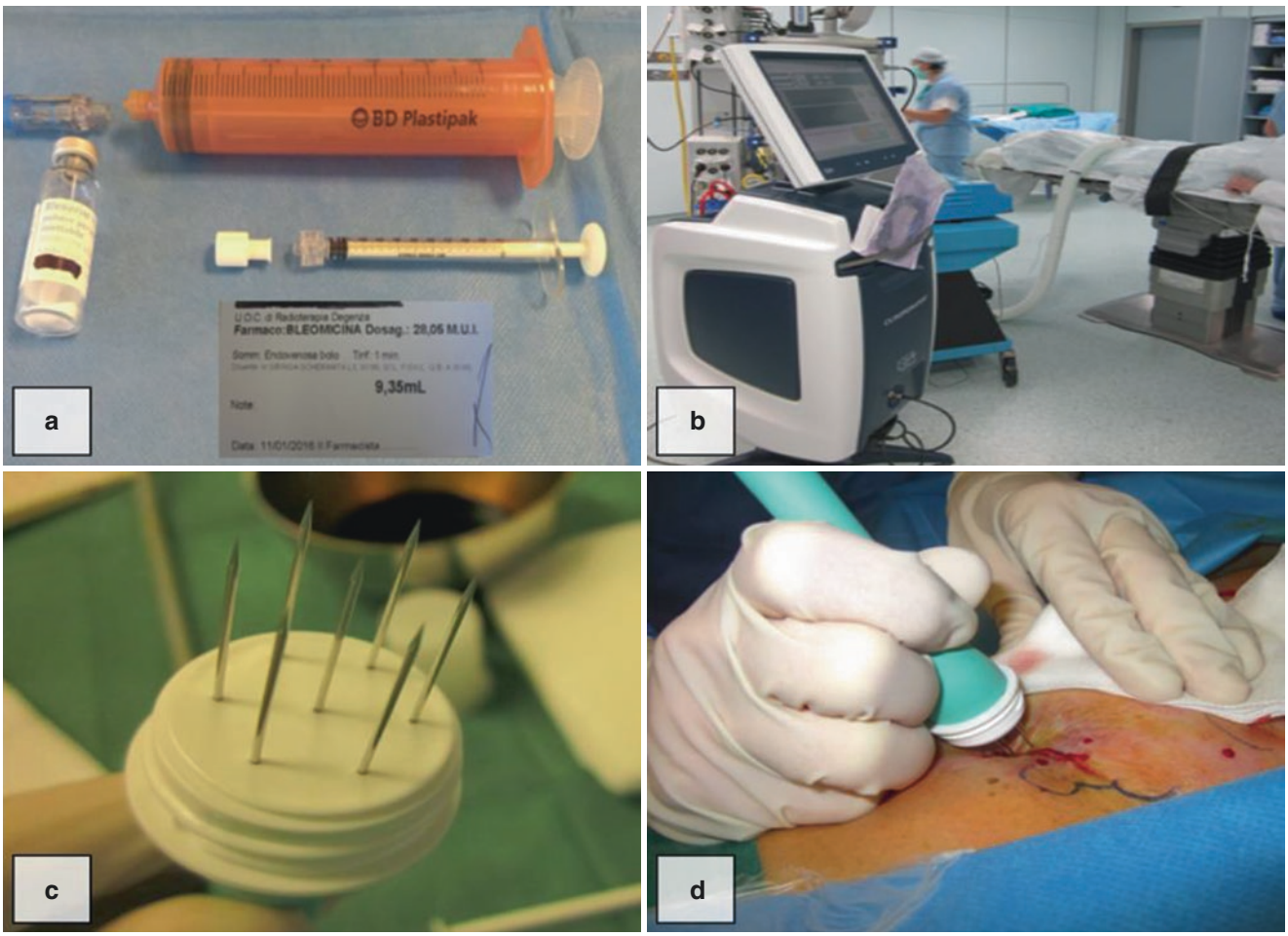

Fig. 76.1 Electrochemotherapy procedure. (**a**) Preparation of cytotoxic chemotherapy: drugs can be administered as either intravenous bolus (large syringe) or direct intratumoral injection (small syringe); (**b**) the electric pulse generator in the operating room; (**c**) an *hexagonal array* electrode; (**d**) electrode application on a patient with chest wall recurrence after mastectomy

76.2.2 Electric Pulse Generators and Electrodes

Nowadays, electrochemotherapy is being used throughout Europe in more than 150 centers with the newly developed Cliniporator™ pulse generator that is CE certified for use on patients (Fig. 76.1b). This electric pulse generator has several advantages that make it a leading product on the market in this area. It generates square-wave electric pulses with variable amplitude and possesses two options for the frequency of the delivered electric pulses (1 or 5000 Hz). The device is computer controller by the operator. In addition, it provides storage of the patient's characteristics as well as of the electrical parameters used for the treatment including traces of voltages actually applied as well as electric current flowing through target tissues during the procedure. Tumors can be effectively electroporated by a train of square-wave electric pulses, usually of the amplitude over distance ratio of 1000–1300 V/cm. During the procedure, each pulse delivery consists of a train of eight electric pulses at 5000 Hz repetition frequency [16]. Delivery of electric pulses can be performed by means of electrodes with different size and geometry. These pulse applicators can be divided into non-invasive, i.e., plate electrodes, or invasive, i.e., needle electrodes [1, 16, 18]. The plate electrodes are intended for superficial tumors and are used by placing tumor tissue in-between their blades, if tumor size is up to 8 mm, or placing consecutively the device more times on the target area, until the whole tumor and safety margins are covered with electric pulses. The needle electrodes are provided with different configurations, according to the specific clinical requirements. Two main electrode types are generally used during breast cancer treatment, the *linear array* electrode and the *hexagonal array* electrode. The linear array electrode is composed of two, 4 mm spaced, rows of 2 cm long needles and is aimed to treat small-sized nodules or tumors confined in a limited area. On the other hand, the hexagonal array needle electrode is composed of seven needles arranged in a hexagonal fashion and can be provided with 2 or 3 cm long needles, according to tumor size and depth (Fig. 76.1c). It is intended at treating bigger and deeper tumors. A single application of the hexagonal electrode covers indicatively an area of 2 cm^2, but during the procedure it can be placed several times on the target area and allows for extensive coverage of tumor-infiltrated skin on the chest wall (Fig. 76.1d).

76.2.3 Anesthesia

Overall, treatment with electrochemotherapy is well tolerated by patients. Nevertheless, the application of the electrodes, especially the needle devices, and subsequent pulse delivery may be two painful procedures, depending also on tumor size and extension. The pain induced by electric pulses is dependent on their amplitude, which in turn is dependent on the distance between the needle electrodes. Therefore, linear array electrodes generally induce less painful pulses for patients. In these cases, only local anesthesia can be used. However, when dealing with patients with multiple tumor nodules or bulky tumors (indicatively larger than 3 cm) or tumors located in delicate or sensitive anatomical areas, general sedation and, in selected cases, general anesthesia represent a reasonable choice. However, also in the rare patients in whom general anesthesia is preferred, the period of assisted ventilation is limited. Generally, posttreatment pain is easily managed with intravenous analgesics during the first hours following the procedure and then by means of oral analgesics. Of course, the intensity of pain treatment depends on the extent of treatment field, its anatomical location, and local conditions of skin and soft tissues, as well as on the electrode type used during the procedure, concomitant sites of disease (e.g., bone metastases), and ongoing pain drugs.

76.3 Indications, Results, and Side Effects

76.3.1 Indications

Electrochemotherapy is currently being used for treatment of cutaneous and subcutaneous metastases from different tumor histotypes. Based on the accumulated experience, accepted indications to electrochemotherapy are the following: (a) curative treatment for patients with multiple skin cancers not amenable with surgery or other local therapies; (b) locoregional treatment of patients with unresectable metastases that are refractory to conventional oncological therapies; (c) exclusive treatment in elderly or frail patients who are deemed not suitable for systemic antineoplastic treatments, radiotherapy, or surgical resection; and (d) palliation of symptomatic (e.g., bleeding) superficial metastases. The first clinical trial was published in 1993 [19], and, since then, several studies have been published, predominantly on malignant melanoma, basal cell carcinoma, head and neck squamous cell carcinoma, and breast cancer. Reported objective response rates range between 72% and 100% for tumors smaller than 3 cm, while significantly decreasing in larger lesions [20–25]. In metastatic patients with skin tumor involvement, the main purpose of a locoregional treatment is not objective response, but to give symptomatic relief in terms of reduced exudate, odor, and bleeding and, ultimately, to preserve patient quality of life. Thanks to its prominent vascular-disrupting action, electrochemotherapy [8, 26] allows to rapidly and effectively manage local bleeding in

patients with ulcerated cutaneous metastases, thus reducing the need of repetitive dressings [27–29]. Furthermore, according to several experiences, electrochemotherapy has proven a reliable palliative tool in the management of painful skin metastases [15, 30, 31] and should therefore be considered a locoregional treatment option that can ensure effective local tumor control and improvement of patient quality of life [23, 32]. Noteworthy, electrochemotherapy has shown activity also in previously irradiated areas and in patients who were refractory to several systemic therapies [15, 32]. Furthermore, it may provide a reasonable alternative in patients in whom surgical resection cannot be attempted or would not be radical. Finally, as opposed to radiation, electrochemotherapy can be safely repeated in order to consolidate tumor response (e.g., to treat patients with partial response after the first cycle) or to treat newly occurred metastases outside the treatment field [22, 23, 32].

76.3.2 Results

According to a large meta-analysis including 44 studies (although mainly performed before the standardization of the procedure in 2006) and 1894 tumors, electrochemotherapy has significantly higher antitumor activity (by more than 50%) than bleomycin or cisplatin alone [33]. Treatment effectiveness was significantly higher for intratumoral than for intravenous administration of bleomycin, while bleomycin and cisplatin administered intratumorally proved to be equally effective when combined with electric pulses. Regardless of drug and route of administration, reported overall and complete response rate were 82.8% and 62.6%, respectively. In a recently published meta-analysis, efficacy of electrochemotherapy in treatment of patients with skin metastases was estimated to be comparable to other skin directed therapies such as radiotherapy, photodynamic therapy, intralesional therapy with antineoplastic agents, and topical therapy [34].

76.3.3 Side Effects

Side effects to electrochemotherapy are generally limited and mild, so that treatment is well tolerated also by elderly and frail patients [35]. Nevertheless, insertion of electrodes and delivery of electric pulses may cause different grades of pain. Moreover, electric pulses may induce an involuntary contraction of underlying muscles, and this may cause an unpleasant sensation for patient, particularly if the procedure is being performed under local anesthesia [15, 36]. Consequently, electrochemotherapy can be administered under either local or general sedation/general anesthesia, according to disease extension, anatomical location, and patient preference. Following the procedure, transient pain and mild inflammatory reaction in the treated field are generally observed. In the following weeks, tissue necrosis and ulceration may occur in a minority of patients. Skin hyperpigmentation can be seen as a well-known side effect of bleomycin, particularly after repetitive treatments [37]. Systemic side effects such as nausea and fever, which are generally transient and mild, are caused by the drugs used during anesthesia and by bleomycin. As a note of caution, electrochemotherapy with intravenous bleomycin should not be used when the cumulative dose exceeds 400 units, due to the risk of pulmonary toxicity (i.e., lung fibrosis) [38]. Posttreatment pain is another crucial issue when considering electrochemotherapy in patients with cutaneous metastases from breast cancer. The management of posttreatment pain may be particularly challenging when dealing with large (>3 cm in diameter) or widespread tumors on the chest wall [24]. Nonetheless, in most patients, posttreatment pain seems to decrease after 45 days according to a retrospective multicenter study conducted on 121 patients with different tumor types [31]. In this study, the authors were able to identify the following parameters as the most reliable predictive factors for posttreatment pain following electrochemotherapy: diagnosis of breast cancer, large tumor size, presence of pain before treatment, and previous radiotherapy. The severity of patient-reported posttreatment pain increased with the increasing number of electrochemotherapy cycles in a phase II study including previously irradiated mastectomy patients [32]. In summary, although electrochemotherapy is a local treatment, which is generally well tolerated by patients, it should be taken into careful consideration that it is not free from possible, sometimes troublesome, side effects. Therefore, it should be clear to the treating physicians that, in patients with rapidly progressive disease, control of cutaneous metastases cannot always be obtained, and if an improvement in symptoms is not reliably expected, treatment should be postponed or avoided. This means that, although expected side effects are few and generally mild in most cases, they should be always carefully weighed against foreseen, realistic benefits. It is advisable that clinicians take into account these elements before recommending treatment with electrochemotherapy and discuss this treatment option in the frame of multidisciplinary meetings (Table 76.1).

Table 76.1 Advantages and limitations of electrochemotherapy

Advantages	Disadvantages
Possibility to be performed under short general sedation (20–30 min)	Treatment-induced pain (depending on disease extension)
Suitable also for frail and elderly patients	Electrode application is operator-dependent
The procedure is easy to perform	Skin hyperpigmentation
Treatment has minor side effects	Possible tissue necrosis
High response rate and limited effect on healthy tissues	Lack of electrodes suitable for targeting tumors with different size, morphology, and location
Short hospital stay required	Possible skin ulceration
Rapid management of symptomatic metastases (painful, bleeding, oozing)	Lung fibrosis (bleomycin-induced, after repetitive treatments)
Preservation of patient function and quality of life	
Favorable cost/benefit ratio	
Treatment	
Reproducibility durable local tumor control	

Table 76.2 Published studies on electrochemotherapy in breast cancer

Author, year	Study	Pts	ECT protocol	Tumor response	Tumor control	Patient benefit	Toxicity
Matthiessen, 2012 [24]	Phase II	17	ESOPE	PR: 33%[a]	nr	Decreased exudate, odor, and bleeding	Local pain
Campana, 2012 [32]	Phase II	35	ESOPE	CR: 54%[b] PR: 37%[b]	3-year, 81%	nr	Local pain, skin toxicity
Benevento, 2012 [41]	Retro	11	ESOPE	CR: 75%[c] PR: 17%[c]	nr	nr	Local pain, skin toxicity
Campana, 2014 [17]	Retro	55	ESOPE	<70 years, CR: 26% [b,d] ≥ 70 years, CR: 57%[b,d]	<70 years, 2-year: 93%[e] ≥70 years, 2-year: 67%[e]	nr	Local pain, skin toxicity
Cabula, 2015 [42]	Retro	125	ESOPE	CR: 58%[b] PR: 32%[b]	1-year, 86%	Predictors for response[e]	Local pain, skin toxicity

[a]Radiological assessment; [b]objective per-patient response; [c]objective per-tumor response; [d]$P = 0.023$; [e]$P = 0.061$ [e]In this study, small tumor size, absence of visceral metastases, estrogen receptor positivity, and low Ki-67 index were predictors of CR after ECT
CR complete response, *ECT* electrochemotherapy, *nr* not reported, *PR* partial response, *retro* retrospective, *tox* toxicity

76.4 Clinical Experiences in Breast Cancer

Several early clinical studies on electrochemotherapy treatment of skin metastases included also breast cancer patients [20, 21, 23, 35, 36, 39]. The results of these preliminary and isolated experiences were summarized in a review, which pointed out that the activity of electrochemotherapy on cutaneous metastases from breast cancer is comparable to that reported in other tumor histotypes [40]. In particular, overall objective response rate in breast cancer patients was 89% (59% complete), and in patients with other tumor histotypes, it was 78% (52% complete). Since 2012, five studies (four from Italy and one from Denmark) with electrochemotherapy in patients with cutaneous recurrences from breast cancer have been published. Two of them were single-center phase II studies [24, 32], while the others were retrospective reports (Table 76.2) [17, 41, 42].

76.4.1 Prospective Phase II Study on Bulky Recurrences

A phase II study from Copenhagen University Hospital, Herlev, was focused on electrochemotherapy treatment of bulky (i.e., larger than 3 cm) cutaneous recurrences from breast cancer in patients who exhausted standard oncologic treatments [24]. Seventeen patients were included between 2008 and 2010. Enrolled patients had a total of 25 metastases whose median diameter was 9 cm (range 3–25). All patients were extensively pretreated: all of them received previous chemotherapy (median number of chemotherapy cycles was 4, range 1–7), 8 hormone receptor-positive patients received endocrine therapy, 5 patients received HER2-targeted therapy, and 16 patients were irradiated on the chest wall. Electrochemotherapy was performed under general anesthesia and, in most cases, using intravenous bleomycin. Five

patients were lost to follow-up due to systemic disease progression, thus leaving 12 patients evaluable after at least 8 weeks. A median of one electrochemotherapy session (range 1–4) was administered, and median duration of the procedure was 23 min (range 10–36). By using clinical examination, the authors observed one complete tumor regression and one partial response. Nonetheless, posttreatment CT scan showed that 4 out of 12 patients achieved over 50% tumor shrinkage. In five of 12 patients, a reduction of exudate from treated metastases was reported. Treatment was well tolerated with local posttreatment pain being the main side effect. The study concluded that electrochemotherapy is an active treatment in a heavily pretreated patient population and ensured appreciable tumor reduction and symptomatic relief.

76.4.2 Prospective Phase II Study on Previously Irradiated Chest Wall Recurrence

A phase II Italian study from the Veneto Institute of Oncology and University of Padova enrolled 35 patients with chest wall recurrence after mastectomy from 2006 to 2011 [32]. Enrolled patients were unsuitable for radical surgery and unresponsive to at least two lines of systemic treatment. Of note, all enrolled patients were previously irradiated for chest wall recurrence, and 85% of them received radiotherapy also at the time of primary breast cancer. The majority of them had hormone receptor-positive tumors (68.6%) and visceral involvement (68.6%). Overall, the authors administered 62 electrochemotherapy cycles (a median of two cycles per patient, range 1–3), and median duration of the procedure was 25 min (range 15–35). Average hospital stay was 1 day (range, 1–3). The procedure was well tolerated, and there were no serious treatment-related adverse events. Early toxicity, which was graded G1–G2 according to the Common Toxicity Criteria v4.0, included transient fever and pain in the treated area, which was easily manageable in most cases, although it increased after the following electrochemotherapy cycles. Late side effects included pain and dermatological toxicity as well. One month after electrochemotherapy, patients reported a "moderate" level of pain in 6%, 13%, and 17% of these after the first, second, and third electrochemotherapy session, respectively. As to dermatological toxicity, G3 skin ulceration was reported by five (14%) and two (6%) patients after 1 and 2 months after electrochemotherapy, respectively. Of interest, three (8.5%) patients reported a G1 transient alopecia, likely induced by systemic bleomycin. A total of 516 metastases were treated (median 15 per patient, range, 1–50), and tumor response was assessed on 196 target lesions. Two months after the first electrochemotherapy, an objective response was observed in 32 of 35 patients (91.4%), and 19 patients achieved complete response. Among the complete responders at first electrochemotherapy, ten patients remained local disease-free at a median follow-up of 32 months. The estimated 3-year local progression-free survival was 81%. However, 23 (65.7%) of 35 patients developed new lesions outside the treatment field. The percentage of patients who developed new lesions was significantly lower in complete responders compared with non-complete responders at first electrochemotherapy (47.4% vs. 87.5%, $P = 0.029$). At the end of the study, 12 out of 35 patients achieved both local tumor control (i.e., complete or partial response on electroporated metastases) and freedom from new lesions on the chest wall. These patients represented the subgroup who achieved an effective chest wall control after treatment with electrochemotherapy.

76.4.3 Retrospective Study on Elderly Patients

In a small retrospective study from the Veneto Institute of Oncology of Padova, 52 breast cancer patients who underwent electrochemotherapy were retrospectively analyzed with particular interest for the clinical outcome of elderly patients [17]. Enrolled subjects were divided in two subgroups according to their age (<70 years or ≥70 years). Overall complete response rate was 40%, but, interestingly, it was significantly higher in the elderly compared to the younger patients (57% vs. 26%, $P = .023$) and in patients with better performance status (PS = 0–1, 53% vs. PS = 2, 21%, $P = .048$). Two-year local progression-free survival among elderly patients was 67%. Interestingly, older women seemed less likely to progress outside the electrochemotherapy field (2-year new lesion-free survival, 39 vs. 30%, $P = .075$), but discontinued treatment more frequently due to impaired performance status ($P = .002$). Finally, treatment-related toxicity was not negligible in this cohort. In fact, local pain was graded ≥3, according to a 10-point visual analog scale, by 16/28 (57.1%) and 8/28 (28.6%) elderly patients at 4 and 8 weeks after electrochemotherapy, respectively; moreover, debridement due to extensive tumor tissue necrosis or skin ulceration was required in 5/28 (18%) of older women.

76.4.4 Retrospective Multicenter Study on Predictive Factors

More recently, 125 breast cancer patients who underwent electrochemotherapy were analyzed in the frame of an Italian multi-institutional retrospective study [42]. Objective response rate was 90.2%, with 58.4% of patients achieving complete response. In multivariate analysis, small tumor size ($P < 0.001$), estrogen receptor positivity ($P = 0.016$), low

Ki-67 index ($P = 0.024$), and absence of visceral metastases ($P = 0.001$) were associated with complete response achievement and could represent useful parameters for improving patient selection in future studies.

76.5 Established Indications in Breast Cancer

Cutaneous metastases occur from breast cancer mainly on the chest wall, but they can be observed, although less frequently, also on other anatomical locations (e.g., contralateral breast, upper arm, shoulder, neck, scalp, abdominal wall) (Fig. 76.2). In some patients, they represent the exclusive site of an isolated locoregional recurrence, while in other patients they occur outside the chest wall. They can be associated with lymph node and/or visceral metastases. In theory, both patients with locoregional recurrence (skin-only recurrence) and patients with metastatic disease associated with skin tumor involvement represent suitable candidates for treatment with electrochemotherapy. Nevertheless, due to the lack of solid clinical evidence supporting its application, at present, electrochemotherapy is not included in the clinical practice guidelines for the treatment of locally recurrent or metastatic breast cancer [43, 44]. Consequently, electrochemotherapy is currently used for the treatment of superficially metastatic breast cancer when (a) cutaneous metastases are not amenable with surgery, (b) there is no space for radiotherapy, and (c) the patient has failed conventional systemic treatments. It is therefore evident that, up to now and in current clinical practice, electrochemotherapy is applied mainly with palliative intent (Fig. 76.3).

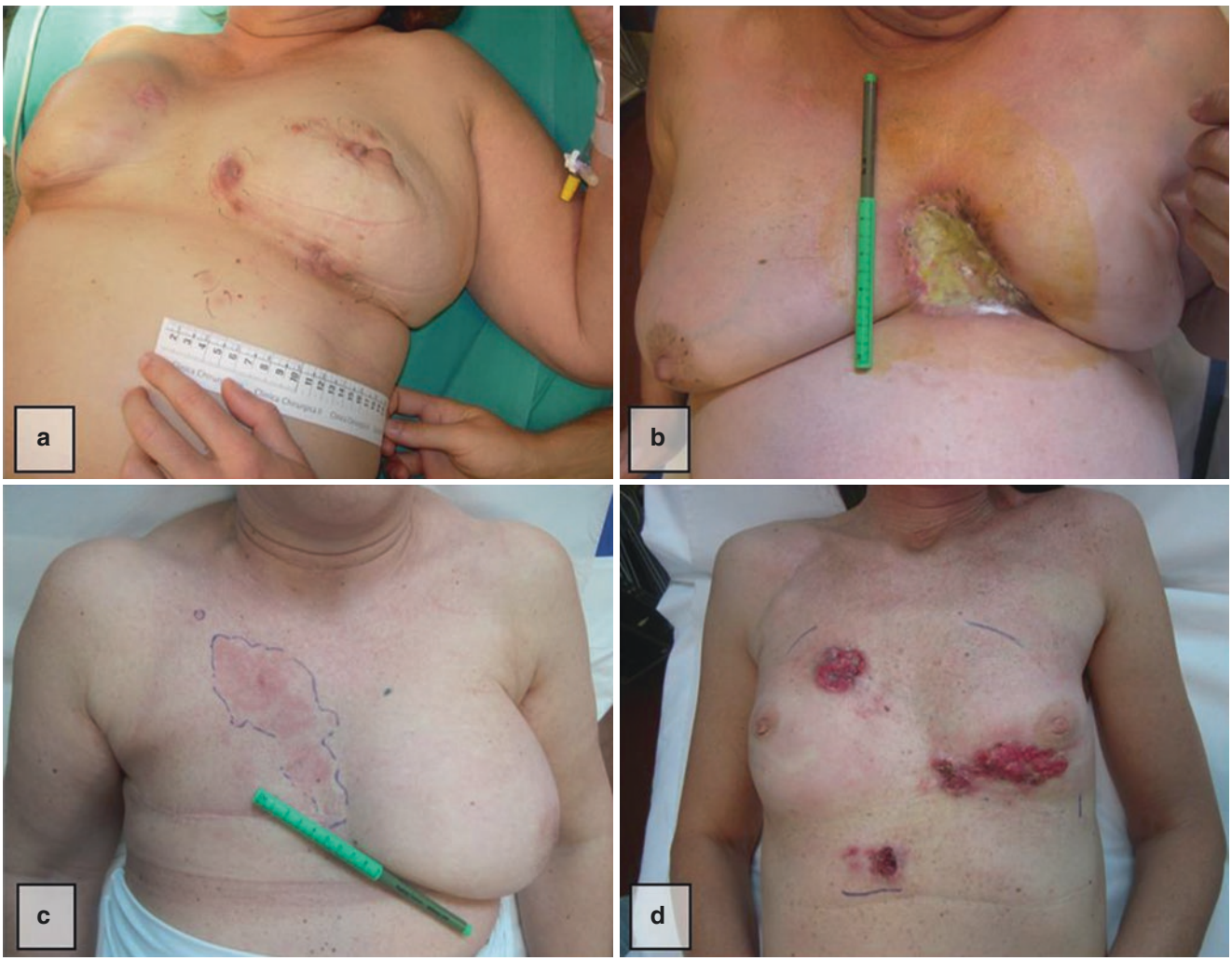

Fig. 76.2 Skin metastases from breast cancer. Different clinical presentations of superficially recurrent breast cancer: (**a**) tumor nodules on the skin of the contralateral breast; (**b**) ulcerated recurrence in the sternal region; (**c**) chest wall recurrence after mastectomy; (**d**) skin tumor involvement of both breasts and abdominal wall

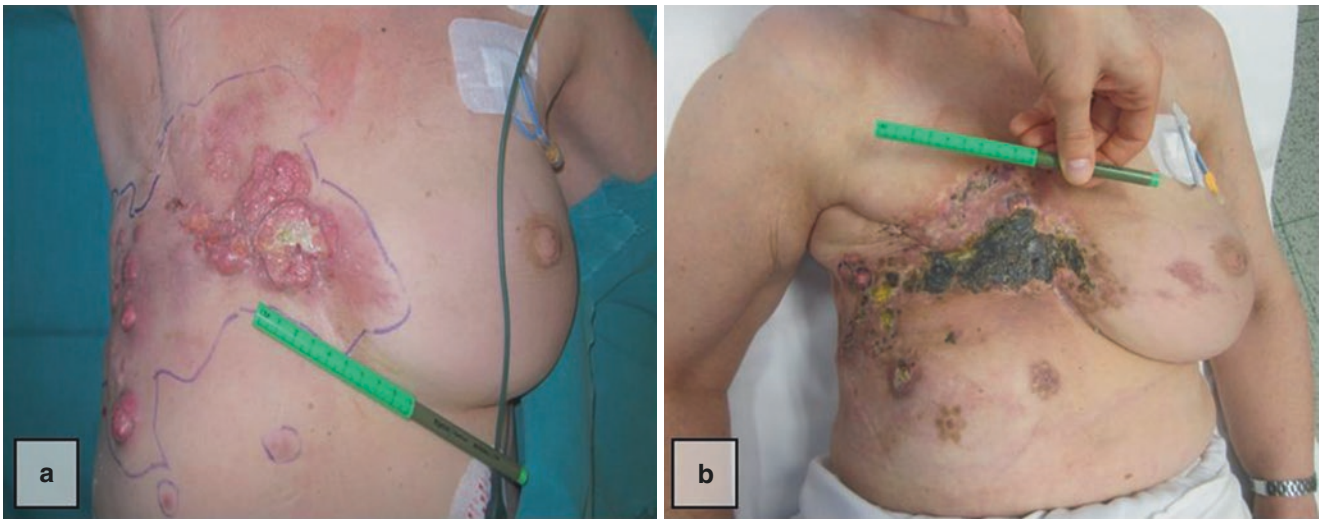

Fig. 76.3 Response to treatment in a breast cancer patient with cutaneous metastases on the chest wall. Preoperative tumor assessment (**a**). Postoperative tumor response (**b**)

76.5.1 Locoregional Recurrence After Mastectomy

Locoregional recurrence after mastectomy (Fig. 76.2c) for invasive breast cancer may be a harbinger of distant metastases and, as such, is generally considered an ominous event. Its occurrence vary widely in the literature, ranging from 5% to more than 40% [45–47]. Also in recent studies and despite widespread use of adjuvant therapies, the occurrence of locoregional recurrence after mastectomy is reported in up to 9% of patients [48]. For patients presenting with locoregional recurrence without evidence of distant metastases, aggressive multimodality therapy is warranted because many of these patients can be rendered disease-free. Therefore, locoregional recurrence after mastectomy, in the form of superficial (i.e., confined to the skin and subcutaneous tissue) locoregional recurrence, represents a suitable target for electrochemotherapy in breast cancer patients. However, in the clinical practice, cutaneous metastases can be observed in very different scenarios. For example, they can occur around the mastectomy scar or, alternatively, on the skin of the contralateral breast. Finally, they can also develop in patients with prosthetic implants; the latter event makes electrochemotherapy particularly challenging due to the possibility of damaging the underlying prosthetic implant with needle electrodes or the possibility of provoking tissue necrosis and skin loss above it [17].

76.5.2 Skin Metastases in Other Anatomical Locations

Breast cancer dissemination can follow either a lymphatic or hematogenous tumor spread. Accordingly, there may be different patterns of superficial tumor dissemination. As a result, lymphatic infiltration can produce large tumor-infiltrated skin areas not only on the chest wall but also on the neck, upper limb, abdomen, etc. Alternatively, discrete tumor nodules may be observed in different anatomical locations as the result of hematogenous dissemination (Fig. 76.2a, b, d). In both cases, cutaneous metastases are the hallmark of widespread tumor dissemination; nevertheless, also in these patients, tumor control remains a fundamental goal because of its potential effect on survival and, most of all, because of its tremendous impact on quality of life. Therefore, electrochemotherapy should be considered if cutaneous metastases are symptomatic (e.g., painful, bleeding, oozing, etc.) and there is the plausible chance to effectively manage them with treatment. In general, when dealing with patients in advanced stages of disease, the value of a locoregional treatment (electrochemotherapy, radiation, etc.) should be thoughtfully balanced against best supportive care, taking into account patient general conditions, life expectancy, disease behavior, superficial disease extension, and location as well as superficial disease-associated symptoms, patient preference, and possible treatment side effects (Fig. 76.4).

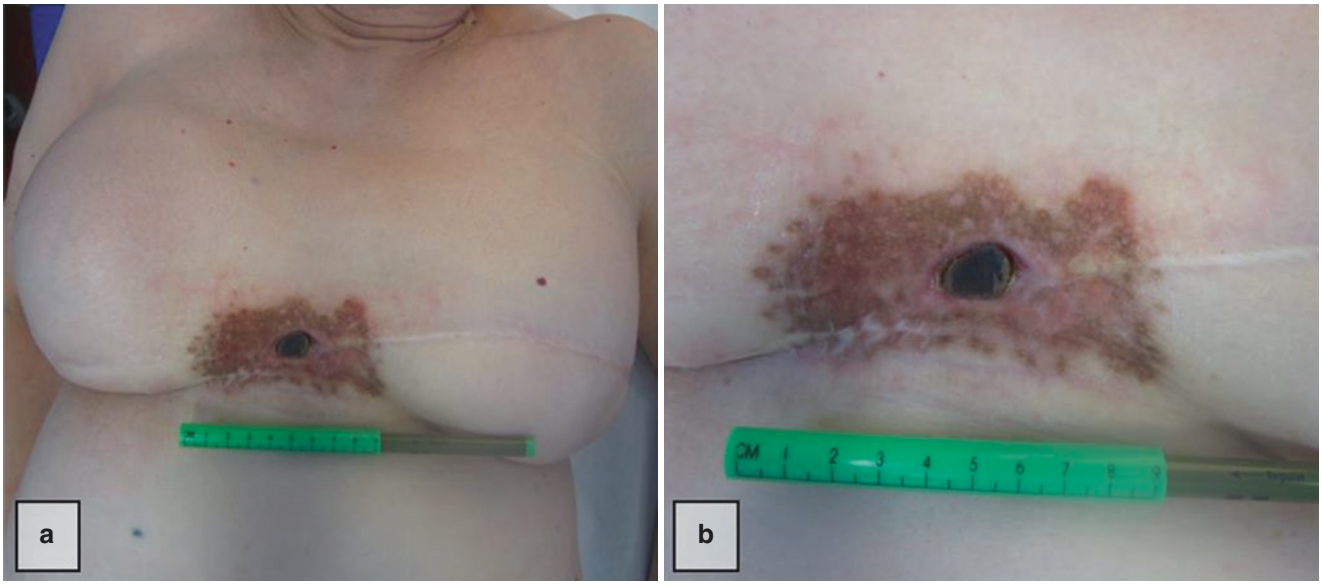

Fig. 76.4 Treatment toxicity. (**a**) An example of coexisting dermatological toxicities following electrochemotherapy. This patient developed both necrosis of tumor tissue, which was easily removed, thus leaving a small tissue ulceration and skin hyperpigmentation in the treatment field (**b**)

76.6 Future Directions

In electrochemotherapy of breast cancer, at least three main research directions deserve investigation in future studies. These are (a) the individualization of new treatment indications (i.e., earlier stages of disease), (b) the combination of electrochemotherapy with systemic treatments, and (c) the development of technological advancements in electrochemotherapy instrumentation aimed at improving treatment delivery and extend treatment indications.

76.6.1 New Clinical Indications

76.6.1.1 Primary Tumors

At present, electrochemotherapy has no indication in the treatment of primary breast cancer. In patients with inoperable, locally advanced disease, electrochemotherapy may be considered as a palliative or neoadjuvant treatment in very selected cases. A single case report has been published so far, where the authors successfully used electrochemotherapy as a neoadjuvant treatment, in order to cytoreduce a large, inoperable locally advanced primary tumor [49].

76.6.1.2 Adjuvant Setting

The sustained antitumor activity of electrochemotherapy makes this therapy a potential valuable option also in the adjuvant setting. In particular, its application may allow targeting minimal residual disease after surgical resection. In particular, when radical resection with clear margins cannot be accomplished, intraoperative electrochemotherapy could be applied to sterilize tumor bed and surgical margins, thus reducing the incidence local recurrence. Although promising in theory, this strategy has not been formally explored, and only a single case report has been published so far, where electrochemotherapy was intraoperatively applied on the tumor bed after tumor resection, in a patient who was unsuitable for adjuvant radiotherapy in order to treat the area at risk of recurrence [49].

76.6.2 Combination with Systemic Treatments

It has been demonstrated that electrochemotherapy is a safe and highly active antitumor treatment; nevertheless, its potential value in breast cancer patients still needs to be explored. Unfortunately, up to now, electrochemotherapy has been mainly used when conventional treatment options

were exhausted and, likely, patients' general conditions were in some way compromised. As a matter of fact, the advanced disease stage and previous extensive treatments likely rendered patient immune system less fit to develop effective antitumor immunity. This hypothesis is corroborated by the absence of any abscopal effect in advanced melanoma patients who were nevertheless successfully treated for superficial metastases by electrochemotherapy [50]. On the other hand, it has been reported that objective response after electrochemotherapy decreases with the increasing size of treated tumors [25]. This indicates that patients with less advanced disease have a higher chance of response to treatment and experience better local control. These observations prompt for considering electrochemotherapy in earlier stages of disease, when tumor size is smaller, and in combination with systemic treatments. Interestingly, electrochemotherapy has been shown to induce extensive tumor necrosis (Fig. 76.3) and to expose tumor antigens [10, 13]. The rational combination with immunomodulating agents may allow capitalizing local immune response and induce a systemic immune response, which could represent an effective strategy to tackle oligometastatic disease. Moreover, since tumor heterogeneity is a common finding in advanced breast cancer patients and mixed response to systemic therapies are commonly observed, the availability of a new, effective, locoregional treatment for superficial metastases may represent a useful tool to combine with or alternate to ongoing systemic therapy, as already suggested in melanoma patients ([51]).

76.6.3 Technological Advancement of ECT Instrumentation (Grid Electrodes, Long Needle Electrodes)

With the adoption of electrochemotherapy by a continuously increasing number of European centers, also the technology has made significant advancements, thus opening the possibility to treat more challenging patients, such as those with widespread tumor dissemination or those with deep-seated metastases.

76.6.3.1 Treatment of Deep-Seated and Visceral Metastases

In recent years, electrochemotherapy equipment has been implemented for the treatment of deep-seated tumors. In particular, thanks to design and development of long (20 cm) needle electrodes, it is now possible targeting a variety of large and deep tumors, including also metastases on soft tissue, bone, and liver [52, 53]. There are several ongoing trials investigating electrochemotherapy, administered by means of dedicated devices, in the treatment of liver and bone metastases [54, 55]. According to the preliminary results of these studies, electrochemotherapy has the potential to spare healthy tissue and holds an exciting potential for the treatment of tumors that lay close to vital structures (i.e., major blood vessels in the liver) where surgery resection is much challenging or not possible [56–58].

76.6.3.2 Treatment of Widespread Superficial Tumors

A subset of patients with locoregional recurrence from breast cancer presents widespread lymphangitic tumor spread on their chest wall. In these patients, tumor recurrence may have ill-defined borders, but the amount of tumor-infiltrated skin is invariably large enough to prevent effective treatment delivery with standard pulse applicators (Fig. 76.3). In order to tackle these challenging situations, some prototypes of a new grid electrode have been recently developed [59]. These new flexible devices adapt to the chest wall and are intended to manage large skin surfaces. This technical advancement may allow homogenizing treatment delivery to the skin and reducing the duration of the procedure, in order to reduce the time of anesthesia, but, most of all, exploit the interval of maximum tumor exposure to drugs.

Conclusions

The treatment armamentarium for superficially metastatic breast cancer has been enriched by the introduction of electroporation technologies in the clinic. Electrochemotherapy represents a promising locoregional treatment modality, which allows effective local drug delivery and concentration within tumors. As a result, clinicians have now the opportunity to apply a rapid, well-tolerated, and effective therapy, which ensures a highly personalized treatment, tailored to the extent of tumor spread. The introduction of a new, effective, and low toxic locoregional treatment, coupled with the advent of new systemic drugs, provides exciting research opportunities to develop novel effective treatment strategies in the challenging setting of locally advanced and metastatic breast cancer. The close collaboration among experts in different fields (medical oncology, surgical oncology, radiotherapy, biomedical engineering) will help to clarify the most appropriate timing and indications for electrochemotherapy and will drive the integration of this therapy in the treatment of breast cancer.

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Circulating miRNA in Early Diagnosis

77

Fabrizio Bianchi

77.1 Background

Breast cancer is the most common female cancer worldwide and the second leading cause of cancer death among women. In 2015, there were an estimated 231,840 new cases and 40,290 deaths in the USA alone [1]. However, mortality can be drastically reduced if this disease is diagnosed at an early stage, before it spreads to other organs. Indeed, the 5-year survival rate for breast cancer patients with such localized disease is ~99% compared to ~25% in patients with metastasis to distant organs (such as to bones, brain, or lungs) [1]. For this reason, there has been a strong drive to promote breast cancer screening programs that allow early-stage diagnosis. This is particularly important in postmenopausal women and in women with multiple cases of familial breast cancer, for whom the risk of developing breast cancer is up to 80% if they are carriers of mutation in BRCA1 or BRCA2 or both [2]. However, there are several drawbacks with this approach: (1) mammography screening is recommended for women over 40 years old; (2) ~20% of tumors will be missed by mammography; (3) cumulative false-positive rates are high leading to overtreatment; and (4) overdiagnosis of tumors that would not have become clinically relevant is common. In addition, the efficacy of mammography screening in reducing mortality is currently debated due to a recently published randomized trial involving ~90,000 women [3–5]. The study showed that breast cancer mortality was not reduced in the mammography-screening arm and that 22% of screen-detected invasive breast cancers were overdiagnosed [3].

The development of cancer biomarkers to be used in addition, or as an alternative, to mammography may change this scenario by increasing the sensitivity (i.e., the ability to detect breast cancer) and specificity (i.e., the ability to exclude the presence of a tumor lesion) of a screening test. Such a test, possibly minimally invasive and relatively cheap, should reduce the size of the target population to be screened and would undoubtedly be advantageous in terms of minimizing harms/maximizing benefits of screening, reducing costs, increasing screening uptake rates, and reducing medicalization of participants.

A blood test for early-stage breast cancer would be ideal as a first-line screening procedure to preselect high-risk individuals who require further diagnostic investigation by mammography or MRI. The relative low-invasive procedure used for collecting material and the possibility to obtain multiple samples from the same patient make liquid biopsy screening (i.e., blood, urine, saliva, etc.) an attractive field for cancer biomarker discovery. Recently, with the discovery of circulating free-microRNA (cf-miRNA), the assortment of molecules analyzable in the blood samples has been expanded. MicroRNAs (miRNAs) are short noncoding RNA molecules functioning as endogenous triggers of the mRNA interference pathway and are involved in the regulation of many cellular processes, including differentiation, proliferation, and apoptosis [6, 7]. MicroRNAs were shown to be remarkably stable in body fluids [6, 8, 9].

There is growing interest in miRNAs, both as potential cancer determinants and biomarkers [10]. Generally, miRNA profiling might be advantageous over mRNA profiling, since the miRNome complexity is approximately tenfold lower than that of the transcriptome (~1900 miRNAs vs. ~20,000 coding genes). This means that a much lower number of samples are needed in the analysis in order to reach a sufficient statistical power. This is relevant particularly in those studies involving human pathological samples, in which genetic heterogeneity among individuals and tumor samples represents a relevant confounding factor. Importantly, the expression of miRNAs is often deregulated in human tumors, both in a tissue- and cancer-specific manner [11].

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77.2 Small Noncoding RNA: The MicroRNA

In 1993, Victor Ambros et al. discovered that the lin-4 (abnormal cell LINeage) gene was involved in the larval development of the nematode *Caenorhabditis elegans* (*C. elegans*), one of the most used model organisms in development biology and genetic research [12]. Lin-4 is a repressor of another gene called lin-14. Lin-14 codes for a protein required for the division of a group of cells during postembryonic development. As opposed to lin-14 gene, lin-4 gene does not encode for a protein but for two small noncoding RNAs [13] measuring 61 nucleotides (nt) and 22 nt, respectively. The longer small noncoding RNA (61 nt) folds into a stem-loop structure that is the precursor of the shorter noncoding RNA (22 nt). This shorter noncoding RNA binds to the 3′ untranslated region (3′-UTR) of the lin-14 messenger RNA (mRNA) through antisense complementarity forming an RNA duplex [13, 14]. This RNA duplex is a signal for the intracellular degradation and translational repression of lin-14 mRNA. Through this molecular mechanism, lin-4 reduces LIN-14 protein level and mediates repression of its biological function [13, 14]. Importantly, lin-4 RNA has been the first identified member of an abundant class of small, regulatory, noncoding RNAs called microRNAs [15–17]. miRNAs have been identified in a wide range of organisms, ranging from simple multicellular, such as poriferans (sponges) and cnidarians (starlet sea anemone), to *Homo sapiens.* Animal miRNAs are supposed to have evolved separately from plant miRNAs due to differences in sequences, structure, and biogenesis mechanisms [18, 19].

77.3 MicroRNA Biogenesis and Function

The mature and active form of a miRNA contains about 22 nucleotides (nt), and it is the result of a complex multistep RNA-processing mechanism which starts in the nucleus and continues in the cytoplasm. MicroRNAs are embedded in an ~33 bp double-stranded stem characteristic of hairpin structures contained in the "precursor microRNA" called pre-miRNA, which can be of several kilobases of length (Fig. 77.1). The transcription of most pre-miRNAs is mediated by RNA polymerase II (Pol II) [20, 21], although a small group of miRNAs that are associated with Alu repeats (i.e., a short stretch of repeated DNA elements) can be transcribed by Pol III [22]. While still in the nucleus, pre-miRNAs are cut at 11 bp from the base of the hairpin stem by Drosha, an RNase III-type endoribonuclease (Fig. 77.1). The result is a shorter RNA with a stem-loop structure of ~70 nt, i.e., the precursor miRNA (pre-miRNA) (Fig. 77.1). Drosha alone is not able to process pre-miRNA, but it works in complex with the dsRNA-binding protein DGCR8 (the DiGeorge syndrome critical region 8, also known as Pasha). DGCR8 works as a "molecular ruler" to guide Drosha cutting. Indeed, mouse embryonic stem (ES) cells deficient in the Dgcr8 gene fail to produce miRNAs and manifest defects in proliferation and differentiation [23].

Subsequently, pre-miRNA is exported to the cytoplasm through the Exportin5-Ran-GTP complex [24] (Fig. 77.1). In the cytoplasm, pre-miRNA is further processed by another enzyme (an endoribonuclease) called Dicer [25, 26]. Dicer belongs to the RNase III protein family and is characterized by a DEXD/H ATPase domain, a DUF283 (a dsRNA-binding) domain, a PAZ domain, an unannotated region ("ruler domain"), and a tandem RNase III domain (RNase IIIa and RNase IIIb) that is the catalytic core [27]. Dicer cleaves the pre-miRNA at a site close to the terminal loop (22 nt away for the dsRNA stem). Following Dicer cleavage, the resulting ~22 nt RNA duplex with protruding 3′ overhangs at both ends (Fig. 77.1) is loaded onto an Argonaute protein (AGO) [28], where one strand, complementary to the target mRNA (guide strand), is selected and subsequently generates the mature miRNA. The mature miRNA-AGO becomes then part, with also the scaffold protein GW182, of the "effector RNA-induced silencing complex" known as miRISC complex (Fig. 77.1) [25, 26]. The "guide strand" directs target mRNA recognition by Watson-Crick base pairing, whereas the other strand of the original duplex (i.e., the passenger strand) is discarded. Argonaute proteins are characterized by the presence of four domains: the PAZ domain (present also in Dicer enzymes), the PIWI domain that is unique to the Argonaute superfamily, and the N and Mid domains. Studies using small interfering RNA (siRNA) duplexes that are similar to miRNA [29] indicate that the relative thermodynamic stability of the two ends of the duplex determines which strand is to be selected [30, 31]. Because strand selection is often not a stringent process, some hairpins produce miRNAs from both strands at comparable frequencies.

77.4 MicroRNA Target Prediction

A significant difference is observed between plants and animals. In the former, targets can be efficiently detected simply by searching for extensive complementarity between the miRNA and 3′ UTR sequences, as shown in [32]. In plants, regulation of transcripts mainly occurs by means of slicing rather than translational repression. In animals, the situation is far more complicated. Extensive complementarity between miRNA and the 3′ UTR sequence exists but is rare [33, 34], and the largest part of validated targets interact with their corresponding miRNA by imperfect match. The initial research effort to reliably identify miRNA targets in animals was heavily based on computational

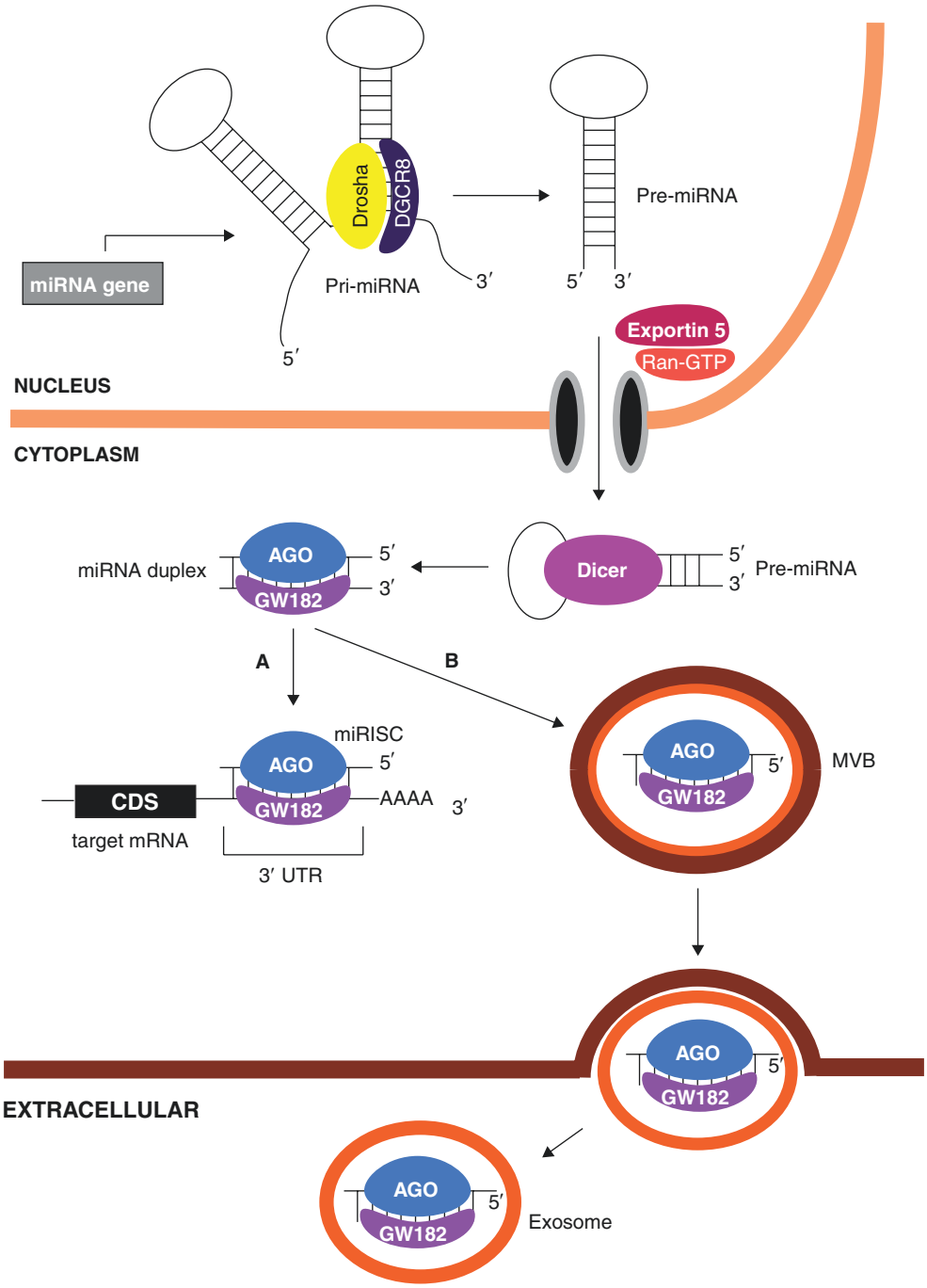

Fig. 77.1 The biogenesis and function of miRNAs. (*A*) Intracellular miRNA mediated silencing of target mRNA. (*B*) Extracellular-vesicle exporting of mature miRNA

tools; thus, a number of different methods were developed. The list of targets produced by these methods, however, showed little overlap which suggests a bias in these results due to intrinsic methodological differences [35]. One of the main problems that occur during miRNA target prediction is the large fraction of 3′ UTR fragments having identical alignment score to the miRNA of interest [36]. Therefore, the inclusion of preferential evolutionary conservation to distinguish a true target from the multitude of equally matching 3′ UTR fragments is considered as mandatory.

Overall, three major aspects should be considered when looking for evolutionary conserved miRNA targets:

1. The seed region, i.e., the 5′ region of the miRNA centered on nucleotides 2–7 [37–41]
2. The conservation of the seed region across different species [38, 42, 43]
3. The observation that highly conserved miRNAs have many conserved targets [38, 42, 43]

In addition, some prediction algorithms (e.g., TargetScan [44]) reward the presence of an adenosine in the "target mRNA" opposite to the first nucleotide (7mer-A1) of the miRNA seed region in order to increase the fraction of true positives findings. This relies on the increasing evidence that a non-Watson-Crick pairing at the first nucleotide of the miRNA is somewhat favored as confirmed both by site-conservation analyses and by array and proteomics data [40, 45]. Another feature considered in some prediction algorithms, although less frequent, is the presence of an additional perfect match in the eighth position (7mer-m8) [35].

77.5 Extracellular MicroRNAs: Mechanisms of Release and Functions

In 2008, pioneer studies demonstrated that microRNAs were detectable in cell-free blood plasma and serum [8, 46, 47]. Other research studies showed subsequently that miRNAs were present in virtually all other body fluids [48–51]. Together these important discoveries posed several questions regarding the way miRNAs remain stable in the body fluids, how they are released from cells, and if they could exert any biological function. Of note, microRNAs were found in the extracellular space within microvesicle/exosome [52, 53], apoptotic bodies (AB) [54], HDL structures [55], or complexed with AGO protein [56, 57], which would confer to miRNA an increased resistance to degradation via RNAse action in the blood [8]. The upload of miRNAs within these structures appears to be guided by either specific or random processes. For example, the AGO-GW182-bound miRNAs were shown to reside in multivesicular bodies (MVB) that give rise to exosomes, which indeed contain high-level of the GW182 protein [58] (Fig. 77.1). This may suggest a random process for miRNA selection within newly formed exosomes, while the observed preferential selection of particular miRNAs can be explained by different decay kinetics [59]. Nevertheless, there are now mounting evidences that positive selection mechanisms do also exist to package specific miRNA species in MVBs [60]. The ceramide-dependent secretory pathway which is controlled by nSMase enzyme (e.g., the ceramide biosynthesis neutral sphingomyelinase) was recently shown to be involved in exosomes and miRNA release [49]. nSMase2 hydrolyzes sphingomyelin to produce ceramide that is essential for budding of intracellular vesicle into the MVBs [61]. Inhibition of nSMase2 specifically reduces miRNA level in secreted exosomes but not the intracellular level of miRNAs [62]. However, how exactly miRNAs are selected and loaded to exosomes/microvesicles, and how the trafficking is regulated in physiological and pathological conditions, is presently unknown. In addition, what is the biological function of these extracellular miRNAs is still an unanswered question. In cancer cells, the extracellular release of miRNAs can be a strategy to reduce intracellular level of miRNAs with antitumor activity [63]. On the other end, the release of miRNAs can function as a paracrine signal to reprogram tumor microenvironment and favor cancer progression. Recently, new evidences favoring this second hypothesis were obtained. Exosomal/MVB/AB miRNAs were shown to be delivered to neighboring cells where, following uptake, they exerted transcriptional modulation of target mRNAs [49, 52, 64–67]. Fascinatingly, an alternative mechanism of action for cancer-derived extracellular miRNAs was also proposed: two independent research groups discovered that AGO2-complexed miR-21 and miR-29a act as signaling molecules via binding to intracellular Toll-like receptors (murine TLR-7 and human TLR-8), which are a family of receptors characteristic of immune cells involved in innate immune system [68]. The activation of immune cells expressing TLRs was shown to be responsible of secretion of inflammatory cytokines that may induce cancer cells spread [68].

77.6 MicroRNAs as Circulating Biomarkers for Early Cancer Detection

Importantly, fluctuations of miRNAs in serum and plasma samples were shown to be associated with many malignant and nonmalignant diseases [8, 69, 70]. Contrary to circulating tumor DNA (ct-DNA), where there are evidences that only a fraction of early-stage tumors may release sufficient quantities of circulating DNA to be detected in the blood [71], circulating free-miRNAs (cf-miRNA) appear to be excellent candidates for blood-borne tumor markers for the early diagnosis of different tumors [72, 73]. Notably, fluctuations in cf-miRNAs have been associated with malignant and nonmalignant diseases [8, 70, 74], which make them excellent candidates for tumor markers for the diagnosis, prognosis, and therapy response prediction.

In line with this hypothesis, our lab and others have recently shown that omics-based analyses of serum/plasma samples are powerful tools for the identification of reliable cancer biomarkers [75, 76]. We successfully derived a cf-miRNA signature capable to early diagnose lung cancer in asymptomatic individuals enrolled in the COSMOS (Continuing Observation of Smoking Subjects) lung cancer early detection trial (ClinicalTrials.gov Identifier: NCT01248806 [77]). From the cf-miRNA analysis, we derived a serum 34-miRNAs signature (Table 77.1) diagnostic for asymptomatic, early-stage, lung cancer (AUC = 0.89, $p < 0.0001$) [75]. The 34-miRNA signature paved the way for the identification of an innovative diagnostic test, the miR-Test (Table 77.1), which once validated in an additional independent cohort of 1115 high-risk individuals confirmed an AUC of 0.85, accuracy of 74.9%, sensitivity of 77.8%,

Table 77.1 cf-miRNA signatures for cancer diagnosis

Study	PubMed ID	*N*	AUC	Serum	Plasma	Scr.	
Bianchi et al.	21744498	34	0.89	√		√	Lung
Montani et al.[a]	25794889	13	0.85	√		√	
Boeri et al.	21300873	13	0.88		√	√	
Sozzi et al.	24419137	24	–		√	√	
Wozniak et al.	25965386	24	0.78[b]		√		
Nadal et al.	26202143	4	0.99	√			
Chen et al.	21557218	10	0.97	√			
Kodahl et el.	24694649	9	0.66	√			Breast
Freres et al.	26734993	8	0.81		√		
Zhang et al.	26476723	3	0.90	√			

Marked cells indicate the kind of samples used to derive the signatures (plasma or serum) and whether the signatures were validated in screening studies. AUC, area under the curve of the receiver operating characteristic (ROC) curve. *N* number of miRNAs in the signature. *Scr.* applied to screening cohorts

[a]miR-Test composition: miR-92a-3p, miR-30b-5p, miR-191-5p, miR-484, miR-328-3p, miR-30c-5p, miR-374a-5p, let-7d-5p, miR-331-3p, miR-29a-3p, miR-148a-3p, miR-223-3p, miR-140-5p

[b]Predicted performance when applied to independent samples

and specificity of 74.8% [78]. Similar studies were performed in breast cancer patients though none of the proposed cf-miRNA signatures have been validated in actual breast cancer screening studies (Table 77.1) [79–84].

Overall, detection of extracellular microRNAs in body fluids is emerging as a promising approach for developing innovative, minimally invasive, cost-effective, diagnostic tests to be used as first-line screening procedures. Several cf-miRNA signatures were recently found and proposed for cancer early diagnosis [76, 78, 85–88], prognosis [89–92], and response to therapy [93–98] and applied in cancer screening studies [78, 87].

Almost half of the published cf-miRNA signatures were discovered analyzing serum samples, while the others were derived from plasma samples (Table 77.1). Serum and plasma cf-miRNAs can be different in terms of quantities and species, and this is ascribed to differences in the chemical composition and in the technical preparation of these two biological specimens [99, 100]. This should be taken into account when searching for overlapping cf-miRNA in different studies or when cf-miRNA biomarkers are validated using external independent cohorts.

77.7 Future Perspective

Liquid biopsies research field is making fast progresses due to the rather simple way to collect biological material and the variety of the analyzable circulating molecules. For these reasons, it is tempting to speculate that liquid biopsies molecular analysis will become the gold standard for developing noninvasive tests for personalized medicine. Population-based liquid biopsies screenings may increase the chance for cancer early diagnosis. In addition, the collection of multiple liquid biopsies from the same patient will allow better evaluation of the therapeutic effect and could anticipate cancer recurrence. Nevertheless, there are still some methodological limitations that need to be resolved before the large-scale application of these molecular tests. For example, the extraction and analysis of circulating nucleic acids require expensive and complex equipment that is currently available only in few cancer centers worldwide. Furthermore, the sensitivity and specificity of these molecular tests should be improved to augment the detection rate and avoid false positives and negative results, which are detrimental for cancer early detection screening programs. Lastly, the possibility to perform molecular tests directly in the blood without nucleic acid extraction by using point-of-care testing (POCT) [101, 102] will definitely facilitate application in a population scale of such molecular tests.

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Relevance of Stem Cells

78

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78.1 Introduction

The incorporation of multidisciplinary care schemes and the introduction of improved chemotherapeutics and molecularly targeted therapies in both the adjuvant and the metastatic setting have significantly ameliorated the clinical management and the survival of breast cancer patients. However, despite these advances, a significant proportion of patients continue to experience therapy failure and disease progression, due to the occurrence of primary or acquired resistance to currently available therapies [1, 2]. Over the last few years, it has become increasingly clear that intra-tumor heterogeneity represents the major underlying cause of disease progression and recurrence following therapy failure in several types of tumors, including breast cancer [3–6]. The tenet of intra-tumor heterogeneity is that the tumor cells that compose the bulk tumor mass, despite their clonal origin, may display a high degree of genetic and phenotypic diversity, which is believed to be at the basis of the emergence of clones of tumor cells that are able to resist to therapies and, eventually, to promote disease recurrence and metastasis [7–9]. This poses a tremendous challenge for the clinical management of breast cancer, in particular with regard to the characterization of biomarkers that can aid prognostic and therapeutic decision-making, and to the development of more effective breast cancer therapies. Here we review the relevance of the cancer stem model to breast cancer and argue that integrating knowledge based on the cancer stem cell paradigm with a better understanding of the molecular alterations underlying breast tumorigenesis holds the key for the development of more refined therapies to combat breast cancer.

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78.2 Cellular and Molecular Bases of Breast Tumor Heterogeneity: The Cancer Stem Cell and the Clonal Evolution Model

The clinical and pathological heterogeneity of human breast cancer has long been recognized [10]. In recent years, molecular profiling studies have tackled the question of inter-tumor heterogeneity in breast cancer, with the recognition of major molecular subtypes (luminal A, luminal B, basal-like, HER2) with different characteristics and clinical behavior [11, 12]. The integration of this new molecular taxonomy with the traditional histopathological parameters (e.g., histological type, grade, estrogen/progesterone receptor, and HER2 status) has revealed critical for informing patient management in the clinical practice. However, converging evidence from a variety of cytogenetic, comparative genomic hybridization and, more recently, parallel sequencing studies support the notion that breast tumors, likewise other solid tumors, rather than being composed of cells with identical functional and phenotypic

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traits, can display varying degrees of intra-tumor heterogeneity, characterized by the coexistence of subpopulations of cells that may profoundly differ in their genomic landscape and behavioral traits [13–18]. These differences exist both in the context of the primary tumor and between the primary tumor and its metastasis [19, 20]. The two models that have been put forward to explain the genesis of intra-tumor heterogeneity are the clonal evolution and the cancer stem cell model [4–6, 21]. There are a number of common features between these two models, and it is becoming increasingly clear that, at least in tumors with high genomic instability, they are not mutually exclusive [3, 4, 20].

Both models hold that tumors originate from single cells that have acquired unchecked proliferative potential and, occasionally the ability to metastasize, through multiple molecular alterations that have enabled tumor cells to break normal tissue constraints. The clonal evolution model, however, posits that tumorigenesis is driven by the clone that has acquired the highest degree of fitness through the accumulation of a series of random genetic and epigenetic alterations, occurred in a spatial and temporal fashion according to a Darwinian evolutionary process [22–24]. In this model, tumor heterogeneity is accounted for by the presence of multiple clones harboring specific genetic and epigenetic aberrations, where one of these clones outcompetes all the others and drives tumorigenesis. In this scenario, also the emergence of therapy-resistant clones, by definition responsible for tumor recurrence and metastasis, becomes the consequence of stochastic molecular alterations that confer a positive selective advantage to specific tumor cells in response to environmental selective pressures, first and foremost anticancer therapies [25, 26]. The therapeutic implication of the clonal evolution model is that eradication of cancer can be achieved only when the entire subpopulation of cells derived from the selected tumor clones are killed by therapies, considering that they all display equivalent degree of fitness and behavioral characteristics.

By contrast, the cancer stem cell model presupposes that tumors are caricatures of normal tissues, and, therefore, the phenotypic and behavioral differences among the tumor cells are the consequence of the hierarchical organization of the tissue generated in the course of a morphogenetic program, albeit aberrant. This model has been validated in several types of solid and hematological malignancies and posits that at the apex of the tumor tissue hierarchy there exist a minority of cells endowed with unlimited self-renewal ability and tumorigenic potential: the cancer stem cells [21, 27–29]. In this model, the bulk tumor population is made of a progeny of tumor cells with only a limited proliferative potential and deprived of any residual tumorigenic ability. The therapeutic implication of this model is that a cure can be achieved when targeted therapies specifically eradicate the cancer stem cells [7, 22, 30]. This concept is of paramount importance in light of increasing evidence that in different types of cancers, including breast cancer, cancer stem cells are inherently resistant to therapies that kill the bulk tumor population, and therefore, able to promote tumor progression and recurrence after a transient response [7, 9, 31, 32].

The clonal evolution and the cancer stem cell models are not necessarily mutually exclusive. Indeed, if it is true that the nature of intra-tumor heterogeneity in the cancer stem cell model is largely phenotypic, recent studies suggest that cancer stem cells may also contribute to the genetic diversity of tumors, due to the presence of cancer stem cell clones that are genetically heterogeneous [13–18]. This can be explained by the high rate of mutations that, in tumors with high genetic instability, may drive clonal evolution of the original cancer stem cell, thus originating a hierarchy of cancer stem cells with varying genetic landscapes. Another possibility is that genetically different clones of cancer stem cells derive from the *de novo* appearance of stemness traits in more differentiated progenitors that have undergone phenotypic plasticity and reprogramming, a phenomenon that, in preclinical studies, has been largely attributed to the activation of epithelial-to-mesenchymal transition (EMT) programs [33, 34]. In keeping with this idea, poorly differentiated breast cancers, which are intrinsically enriched in cancer stem cells [35], frequently display increased mesenchymal-like features [36], and the occurrence of mesenchymal phenotypes has been associated with resistance to hormonal and chemotherapeutic treatments [30, 37–39]. Whichever the underlying cause, the emergence of genetically different cancer stem cell clones would be unavoidably associated with the coexistence of multiple genetically distinct lineages of tumor cells, a scenario that is typical of polygenomic breast cancers [14, 18].

The idea that integrating the cancer stem cell and the clonal evolution models may provide a more comprehensive understanding of tumorigenesis, with profound clinical implications, is of the utmost relevance to breast cancer. On the one hand, the degree of molecular resemblance to stemness traits may constitute a measure of the molecular, biological, and clinical heterogeneity of breast cancers, as indicated by the notion that the biological aggressiveness of breast cancers can be predicted by their intrinsic content in cancer stem cells [35]; on the other hand, recent parallel sequencing studies have demonstrated that breast tumors may display varying degrees of intra-tumor genetic heterogeneity [14, 15, 17], which can even affect well-established driver somatic mutations, such as those involving TP53 and PIK3CA [18]. As to this latter observation, while it has not yet formally proven that this is the consequence of the coexistence of multiple genetically distinct cancer stem cells, it has been demonstrated that either a spatial heterogeneity, with different lineages present across different geographical

areas of the primary tumors, or a temporal heterogeneity, with genetically distinct clones between the primary tumor and its metastasis, is both a possible occurrence in breast cancer [14–16, 40].

78.3 Stem Cells in the Normal Mammary Gland and in Breast Cancer

In normal tissues, stem cells endowed with unique self-renewal and multi-lineage capacity fuel the process of tissue morphogenesis in which stem cells retain their immature state and give origin to progenitors that progressively lose their proliferative ability to undergo terminal differentiation [21]. The concept that tumorigenesis can be regarded as to normal morphogenesis gone awry, with the corollary that tumors are hierarchically organized similarly to normal tissues, suggests that normal and cancer stem cells may share several similarities [6, 27–29, 41, 42]. Indeed, a number of common phenotypic and functional properties have been described between normal and tumor stem cells, such as the ability to self-renew and differentiate into multiple lineages, thus recapitulating the phenotypic heterogeneity of the original tissue, the telomerase and the antiapoptotic activity, the increased membrane transporter activity, and the ability to resist to genotoxic stresses [4, 41, 43]. Moreover, evidence in support of the existence of cancer stem cells in several types of solid cancers, including breast cancer, has been provided through the use of the same *in vitro* surrogate assays, such as sphere formation in anchorage-independent conditions, differentiation and clonogenicity assays, and *in vivo* transplantation techniques (limiting dilution xenografting in orthotopic or heterotopic sites) adopted to study the biology of normal stem cells [4, 44, 45]. Further supporting the idea of the similarities between normal and cancer stem cells, the prospective isolation of rare subfractions of tumorigenic cells from the bulk tumor mass has often been based on the use of cell surface markers derived from the characterization of the normal stem cells in the tissue of origin [29, 35]. The idea of the congruence between normal and cancer stem cells is relevant to breast cancer, where the use of the molecular profile of human normal mammary stem cells has revealed a useful tool to interrogate the molecular, phenotypical, and clinical heterogeneity of breast tumors, with the observation that their intrinsic biological aggressiveness is a function of their content in cells displaying stemness traits [35].

This notwithstanding, it has to be clearly stated that the cancer stem cell concept does not necessarily address the original cellular target of malignant transformation. The mammary gland constitutes a paradigmatic model in support of this concept. In fact, while it is true that—as shown in preclinical models of breast tumorigenesis—breast cancer stem cells may derive from alterations of the homeostasis of normal mammary stem cells [34, 46], it has also been shown that cells with tumorigenic ability may originate from immature progenitors and terminally differentiated cells reprogrammed to a stem cell-like state through phenotypic and molecular plasticity consequent to the activation of an EMT program [34, 47]. This notion is in keeping with the well-established role of EMT in the transdifferentiation of normal mammary epithelial cells to a more mesenchymal state [33] and with evidence implicating EMT in therapy resistance and cancer progression [39, 48, 49]. Therefore, the concept of cancer stem cell is rather an operational definition that, irrespective of the cell of origin, indicates—in the context of the hierarchical organization of breast tumors—the true underlying cause of the tumorigenic process and, most likely, the ultimate responsible for tumor progression and metastasis after therapy failure.

The relevance of the cancer stem cell paradigm to breast cancer is also underscored by accumulating evidence that cancer stem cells are inherently resistant to conventional chemo- and radiotherapies, thus being able to drive tumor progression and recurrence even when standard of care treatments have yielded an initial response in terms of debulking of the primary tumor. While data are emerging in support of this view [7, 30, 37–39], the mechanisms underlying the therapeutic resistance of breast cancer stem cells remain elusive. A number of properties likely contribute to the emergence of therapy-resistant breast cancer stem cell clones, such as the high capacity for DNA repair, the relative dormancy/slow cell cycle kinetics, and the expression of multiple drug resistance membrane transporters (e.g., ABC transporters) and of anti-apoptosis determinants. These mechanisms have been, therefore, proposed as potential therapeutic targets to eliminate breast cancer stem cells and to achieve a definitive cure [9].

78.4 Molecular Mechanisms Underlying the Origin of Breast Cancer Stem Cells

The mammary gland represents a paradigmatic example of how the coordinated execution of normal tissue morphogenesis represents *per se* an effective tumor suppressor barrier. Over the past decade, evidence has accumulated that hijacking the developmental pathways that control the self-renewal of normal stem cells and tissue morphogenesis (Notch, Hedgehog, and Wnt) can be involved in breast tumorigenesis [50–55]. Therefore, much interest has grown concerning the possibility that targeting these pathways, in combination with conventional anticancer treatments, could improve the clinical management of breast cancer [41, 56–59].

The intimate relationship between deregulation of the mechanisms that tightly regulate normal mammary morphogenesis and onset of breast tumorigenesis is epitomized by the

Numb-p53 tumor suppressor circuitry. Dysfunction of this functional axis results in the emergence of cancer stem cells at multiple levels of the mammary tissue hierarchy [34]. At the level of the mammary stem cell compartment, proficiency of the Numb-p53 circuitry is required for the correct execution of asymmetric cell divisions by normal mammary stem cells [34, 46]. Asymmetric cell division is the process through which, at mitosis, a normal mammary stem cell self-renews and withdraws into quiescence while originating a progenitor that actively proliferates in the transit-amplifying compartment and is fated for terminal differentiation [60]. The ability to generate two daughter cells with opposite proliferative and differentiative fates through asymmetric cell divisions is vital to prevent the uncontrolled expansion of the stem cell compartment. The regulatory function exerted by Numb, by the liaison of p53, over this process safeguards against the emergence of cancer stem cells with unlimited proliferative and high tumorigenic potential [34]. The Numb-p53 circuitry, however, also counteracts the appearance of cancer stem cells at the bottom of the mammary gland hierarchy, in the compartment of differentiating progenitors and terminally differentiated cells. At this level, Numb-p53 dysfunction disrupts proper progenitor maturation and induces, through EMT activation, reprogramming and dedifferentiation of progenitors to a stem-like state, a process that is also accompanied by acquisition of tumorigenic potential [34]. Remarkably, restoration of the proficiency of the Numb-p53 circuitry efficiently curbs tumorigenesis, an effect that depends on the selective targeting of breast cancer stem cells, in the absence of a significant impact on the bulk tumor population [34]. These findings represent an important proof of concept that the elucidation of the molecular mechanisms responsible for the appearance of cancer stem cells may have an important impact on the development of new specific anticancer stem cell therapeutics that, integrated with conventional chemo- and radiotherapies, may significantly aid the clinical management of breast cancer.

78.5 Use of the Cancer Stem Cell Concept to Drive the Discovery of Targeted Therapies: The Paradox of Response and Survival in Cancer Therapeutics

The concept that cancer stem cells may represent the Achilles heel of tumors has also profound implications for the rationale designing of new anticancer drugs. Presently, evaluation of the efficacy of new cancer therapeutics in clinical trials is largely based on the objective clinical response, i.e., the assessment of changes in the bulk tumor mass according to RECIST (Response Evaluation Criteria in Solid Tumors) criteria, achieved by direct measurement or diagnostic imaging [61]. The principle at the basis of this approach is that objective evidence of clinical response, which can be assessed over weeks to months, constitutes a surrogate for biological activity and clinical benefit. By contrast, disease-free survival, which would be the ideal clinical endpoint to establish the efficacy of a cure, would require long-term follow-up. There is, however, increasing concern that assessment of the short-term effects of a drug in phase 2 trials using RECIST criteria might underestimate the effects of compounds with a selective anticancer stem cell action [9]. Indeed, the short-term reduction in the tumor mass largely reflects the impact of a given drug on the bulk tumor population. By contrast, a drug with a selective level of action against cancer stem cells may display only a modest, if any, effect in short-term treatments, while it could show a clinical benefit in terms of long-term survival. The idea that current drug development designs are more suited for the selection of therapeutics directed against the bulk tumor population, rather than against cancer stem cells, might also explain why, in a variety of hematological and solid malignancies, including breast cancer, the initial clinical response does not necessarily translates into increased patient survival [62]. While using disease-free survival as a primary clinical endpoint to assess the efficacy of a new drug against cancer stem cells is impractical, the task of translating new drugs to the clinical practice should integrate new preclinical and clinical methodologies that include the cancer stem cell concept. One possibility is, for instance, the routine implementation of neoadjuvant clinical trials (where a complete pathological response is a reliable surrogate for reduced recurrence rate) in which the objective response is integrated with cancer stem cell-based biological endpoints (biomarkers or functional proxies *in vitro* and/or *in vivo*) assessed on biopsy specimens obtained before and after therapy.

78.6 Concluding Remarks

The cancer stem cell paradigm is destined to profoundly revolutionize our way to diagnose, prognose, and cure breast cancer. While surgery and adjuvant therapies remain the standards of care for the treatment of breast cancer, the integration of our increasing understanding of the genetic and epigenetic bases of breast tumor heterogeneity with a more complete understanding of the cancer stem cell biology is likely to provide in the forthcoming future new therapeutic strategies to combat breast cancer and to overcome therapy resistance and tumor progression that often detrimentally affect breast cancer patient survival.

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Part XII

Biostatistics and Bioinformatics

Subgroup Analyses and Information from Clinical Trials on Breast Cancer

79

Wai-Ki Yip

79.1 Introduction

At the 14th St. Gallen International Breast Cancer Conference in 2015, a panel of breast cancer experts reviewed and endorsed evidence-based local and regional treatments for early breast cancer [1], drawing on the research presented at the conference and identifying therapeutic targets based on disease heterogeneity.

As evidenced in the St. Gallen Conference, medical research is now more focused on providing personalized care for patients, which requires investigating how patient characteristics, including novel biomarkers, modify the effect of current treatment modalities. This phenomenon is known as heterogeneity of treatment effects. A better understanding of the interaction between treatment and patient-specific prognostic factors will enable practitioners to expand the availability of tailored therapies, with the goal of improving patient outcomes. But, how do we collect and analyze heterogeneity information?

79.1.1 An Example of Traditional Subgroup Analysis in Clinical Trials: The HERA Trial

The HERA (Herceptin Adjuvant) trial is an international, multicenter, randomized, open-label, phase 3 trial comparing treatment with trastuzumab for 1 and 2 years with observation following standard neoadjuvant, adjuvant chemotherapy, or both in patients with HER2-positive early breast cancer. Trastuzumab's benefit was first reported in 2005 [2] and was further documented in two follow-up analyses [3, 4] demonstrating that treatment with adjuvant trastuzumab for 1 year following chemotherapy provided significant clinical benefits (disease-free survival, overall survival) for patients with HER2-positive breast cancer. The HERA trial used a randomization procedure with stratification according to region of the world, age, nodal status, type of chemotherapy, and hormone receptor status together with intention to use endocrine therapy. Figure 79.1 shows the forest plot of an exploratory disease-free survival subgroup analysis comparing 1 year of trastuzumab versus observation, at 2 years "median" follow-up.

The forest plot [5, 6] is a popular graphical way to present the result of an exploratory subgroup analysis from a randomized clinical trial (RCT). Note that the overall result from the trial is positive (with hazard ratio [HR] = 0.64) and the main objective is not to test the null hypothesis within each subgroup but rather to assess heterogeneity around the overall effect which is indicated by the *solid vertical line* in Fig. 79.1. This (marginal) subgroup analysis was prespecified in the protocol and Cox proportional hazards models including interaction terms for treatment and each indicator of the individual subgroup factor were computed. Thus, visually, the forest plot summarizes the heterogeneity of treatment effects in all the subgroups for all prespecified factors in a single plot. The solid vertical line represents the overall hazard ratio (HR) comparing trastuzumab versus observation. The dotted vertical line is at HR = 1 which represents the null hypothesis of no effect. From the forest plot, the HRs for the different subgroups of the nodal status (positive, negative) were very similar (homogeneous). However, the trastuzumab effect for patients enrolled from Central and South America appears to be less than for patients from other regions of the world. How does one interpret such results from the subgroup analysis? Are the results trustworthy? Should regulators and/or clinicians recommend different treatments for, say, different regions of the world based on these results? Note that similar questions may be asked about very large tumors (>5 cm) or older patients (>60 years old). Additional subgroup analyses using some of the proposed methods here may help to answer some of these questions.

As the confidence intervals of all the subgroups in the forest plot include the solid vertical line, none of the

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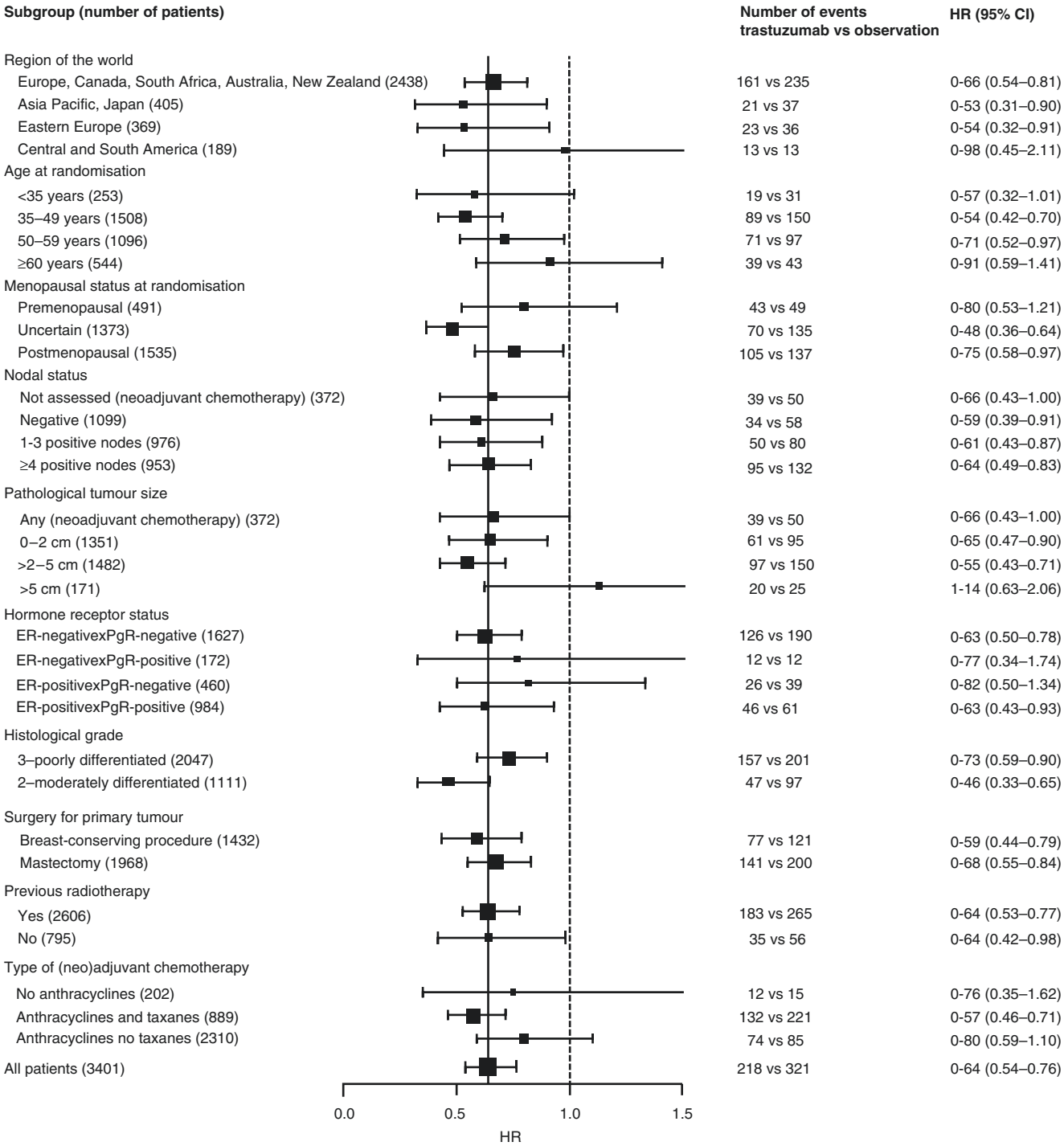

Fig. 79.1 Forest plot of subgroup analysis for 1 year of trastuzumab versus observation with disease-free survival as outcome [3]. Reprinted from The Lancet, Vol. 369 Smith I., et al., 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomized controlled trial. pp. 29–36. © (2007), with permission from Elsevier

heterogeneities shown were statistical significant. However, some of them warrant further investigation. In the later section entitled Tools for subgroup analysis, we describe a number of statistical methods that one could use to analyze and explore these treatment effect heterogeneities.

79.2 Subgroup Analysis and Breast Cancer

Results from RCTs provide the foundation of evidence-based medicine by comparing the treatment effects of competing therapies. Assessment of effectiveness is generally

based on the entire cohort of patients enrolled in the study. As shown in the HERA analysis, the magnitude of the treatment effect may not be the same across different patient subpopulations. Thus, the traditional one-size-fits-all treatment recommendation may not be optimal for an individual patient. Evaluating the interaction between treatment group and patient characteristics may provide the information necessary for clinicians to customize treatment to individuals and maximize the treatment benefits. For example, if the overall trial results fail to show significant treatment effects between new and conventional treatments, the new therapy may still be better in certain patients in particular subgroups.

A common approach is the one illustrated above, i.e., to evaluate the treatment effects for specific end points for subgroups of the patient population defined by baseline characteristics. An "interaction" statistical test of the null hypothesis that the treatment effects are the same in these subgroups can then be performed. Heterogeneity is classified as quantitative when the new treatment is superior across subgroups but the magnitude of the benefit differs and is classified as qualitative when the treatment is superior in one subgroup but has no effect or is inferior in another subgroup. Clinically, the qualitative interactions are more interesting. This approach to the study of treatment effect heterogeneity is known as "subgroup analysis" [7].

Breast cancer is a very diverse and complex disease. We have yet to understand fully the causes and the underlying biological mechanisms involved in the disease. There are a number of prognostic factors that could affect the disease outcome. By analyzing subgroups of patients with different prognostic factors, we may be able to identify specific subgroups which may respond well to different treatments. Hence, research results from subgroup analyses could allow clinicians to customize treatments for breast cancer patients for better outcomes.

79.3 Breast Cancer Heterogeneity

Subgroup analysis should be carefully planned and justified. Although the determination of pathophysiological heterogeneity is needed, Rothwell [8] has suggested four additional indications for subgroup analysis. They are discussed below with examples from breast cancer studies. Note that these indications are not mutually exclusive as some of these indications can always be traced to some underlying physiological conditions. The investigation of the following types of heterogeneity in breast cancer may provide necessary clinical information for treating an individual:

1. *Heterogeneity related to risk*—Clinically important heterogeneity of treatment effect is common when groups of patients have different absolute risks with or without treatment or with two or more evaluated treatments. The need for reliable data regarding risks and benefits in subgroups and individuals is greatest for potentially toxic interventions, such as cytotoxic therapy, which provide overall benefit but can harm a proportion of patients. Thus, the probable balance of risk and benefit in individual patients needs to be assessed. Subgroup analysis and risk models can be the correct tools to use. For example, because the risk of recurrence is higher for patients with more positive lymph nodes, the absolute benefit of trastuzumab is greatest in HERA for the 4+ nodes subgroup despite similar relative risk reductions across nodal subgroups (Fig. 79.1).
2. *Pathophysiological heterogeneity*—Subgroup analyses are most informative when they are based on prospectively defined, biologically based assumptions. Differences between groups of patients in underlying pathology, biology, or genetics can each lead to clinically important heterogeneity of treatment effects. Better understanding of the underlying molecular mechanisms of the disease will lead to better treatment. Clinicians often have to treat patients with ill-defined clinical tumors, which probably have many underlying pathologies, rather than one disease. At the 13th St. Gallen conference [9], there were efforts to characterize tumor subtypes and to classify breast cancer as luminal A, luminal B, Erb-B2 overexpressing, and basal like. Different therapies were recommended for patients according to these subtypes. Subgroup analyses can also be useful when there are predictable differences in the biological response to the underlying disease. Genotype is an important determinant of both the response to treatment and the susceptibility to adverse reactions for a wide range of drugs. The mutations of BRCA-1 and BRCA-2 genes may affect the prognosis. Additional biomarkers such as Ki-67, status of ER, nodes positive/negative, and HER2 help to identify optimal treatment regimes. In the PACS01 trial, it was suggested that Ki67 is a biomarker candidate for predicting docetaxel efficacy in ER-positive breast cancer [10]; in the BIG 1-98 study, it is suggested that patients with higher Ki-67 values who were assigned to receive tamoxifen had poorer prognosis compared with letrozole [11]. Using information from gene expression profiles such as the 21-gene RS [12, 13], the 70-gene signature [14], or PAM-50 [15] to name a few, provides prognostic and perhaps predictive information regarding the utility of cytotoxic therapy. Although the molecular gene scores provide useful prognostic information, they can be too expensive to be applied in developing countries.
3. *Heterogeneity related to practical application*—The main potential of subgroup analysis is in answering practical questions about how treatments should be used most

effectively, such as at what stage of the disease is treatment most effective, how long a particular therapy should be applied to achieve optimal results, or how the risks and benefits are related to comorbidity. The effect of treatment is often critically dependent on its duration. The optimal duration of adjuvant tamoxifen was addressed by the ATLAS study, which suggested a significant benefit for extending such treatment to 10 years rather than 5 years after the diagnosis of estrogen receptor-positive breast cancer [16]. A joint analysis of two randomized trials (International Breast Cancer Study Group [IBCSG] VI and German Breast Cancer Study Group) concluded that three initial cycles of adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy were as effective or ineffective as six cycles for older premenopausal women (≥40 years old) with ER-positive tumors [17]. For node-positive breast cancer, a number of analyses suggest the efficacy of adjuvant docetaxel in several breast cancer subtypes [18–20]. Less invasive and less expensive treatment may be more appropriate for early-stage breast cancer. For example, two large studies support the safety and efficacy of shorter courses of whole breast radiation therapy (40 Gy in 15 or 42.5 Gy in 16 fractions) which offer advantages of convenience and cost compared with the previous standard of 50 Gy in 25 fractions [9].

4. *Underuse of treatment in specific groups*—Treatments that are effective in trials are often underused in specific groups of patients in routine practice. For example, selective estrogen receptor modulators (SERMS) such as tamoxifen were not used in younger patients for many years. In general, older patients and minorities have been underrepresented in clinical trials and more attention is needed so that these groups can be included in trials to determine the effectiveness of treatments for them. Proof of the generalizability of benefit is another major function of subgroup analyses.

79.4 Methodological Issues Concerning Subgroup Analysis

Performing subgroup analysis is a challenging task as documented by many researchers [7, 8, 21]. In fact, many examples of subgroup analyses that suggested clinically important heterogeneity of treatment effects have subsequently been shown to be false. For example, a published report in the New England Journal of Medicine claimed that tamoxifen citrate was ineffective for women aged <50 years with breast cancer [8]. This result was refuted in subsequent research [22]. As statisticians have pointed out, many such analyses have been overinterpreted and led to further research that was misguided or led to suboptimal patient care [8].

Subgroup analysis is inherently problematic. The first problem is due to multiplicity of testing, as it is a common practice to perform multiple subgroup analyses. Thus, the probability of a false-positive finding (type 1 error) increases as the number of subgroup analyses increases. If one performed just 14 independent analyses, there would be a 50% chance of getting a p-value ≤ 0.05 in at least one of them, even if there were no treatment effect at all. While subgroup analyses on the same data are not independent, the principle remains valid that the probability of at least one false-positive signal across all analyses will tend to be inflated. Thus some researchers recommend not presenting the p-values for within-subgroup comparisons but rather to give an estimate of the magnitude of the treatment differences and a corresponding (marginal) confidence interval. In the HERA trial, ten prognostic factors were examined. The effective type I error (probability of declaring significance when there is no real heterogeneity) can be computed under independence as being $(1-(0.95)^9) = 0.40$ if the α level for each separate test is set at 0.05.

In addition, any subgroup analysis, by definition, is not powered to detect the magnitude of effect anticipated for the trial, as the size of the subgroup is smaller than the entire cohort. So even if the overall result is significant, the test conducted for the subgroup may not have enough power to detect a significant result.

The biggest problem with subgroup analyses are their post hoc nature (also known as data dredging or fishing [23]). Post hoc analyses refer to those in which the hypotheses being tested are not specified prior to examination of the data. They are likely to be driven by trends seen in the data. Often, it is unclear how many post hoc subgroup analyses were performed. Thus, the type I error rate can be substantially higher than the nominal rate of 0.05. Since most publications contain only significant results, a significant report from one of these post hoc analyses would therefore potentially lead to misguided recommendations. Ideally, subgroup analyses should be prespecified and documented in the study protocol or at least prior to the examination of any of the data. If p-values are presented, the results need to correct for multiple testing. All the subgroups being analyzed in the HERA trial were prespecified in the protocol; however, after a simple Bonferroni correction, none of the individual subgroup results would be significant.

Although subgroup analyses have certainly led to mistaken clinical recommendations and possible harm to patients, it is imperative to glean the correct information from subgroup analyses because not doing so may also be harmful [8]. To counter some of these problems, the investigators should plan subgroup analyses in trial design by identifying prognostic factors that could potentially interact with treatment as secondary end points and designating their analyses as "exploratory." They must refrain from conducting

"data dredging" as it will most likely result in a false-positive outcome, misguiding future research. Subgroup analyses must also follow stringent reporting guidelines.

Ultimately, if certain prognostic factors are identified as interacting with treatment effects in exploratory analyses, a properly designed and adequately powered trial should be conducted to detect them reliably or pooled meta-analyses of several trials should be undertaken to confirm the result.

79.5 Practical Issues Concerning Subgroup Analysis

Besides some of the inherent methodological issues, there are practical issues when one performs a subgroup analysis.

79.5.1 Definition of a Subgroup

The problem starts with how to define a subgroup. A subgroup is based on one or more patient baseline characteristics that may be categorical or continuous.

(a). Categorical—Some subgroups can be well defined. For example, patients can be identified as male or female. However, it is not easy to define some of the seemingly well-known subgroups. For example, in breast cancer, women whose disease has positive estrogen receptors (ER+) respond well to endocrine therapy, while those with ER disease do not [24]. Similarly, women with human epidermal growth factor receptor 2 disease (HER2+) respond well to anti-HER2 therapy such as trastuzumab [2–4]. But, it is not easy to determine a universally accepted cut point for ER+ versus ER− [25] and also for HER2+ versus HER2− [26, 27].

(b). Continuous—Sometimes the boundaries of subgroups are not clear, especially if the characteristics are measured on a continuous scale. For example, age groups can be categorized in many ways. Sometimes, a cut point is created for convenience or size of populations instead of for definition of meaningful clinical subgroups. However, the specific categorization may affect whether or not a treatment effect is suggested. An example from the IBCSG Trial IX [28] illustrates this situation. This study is the largest randomized clinical trial comparing three courses of CMF chemotherapy followed by up to 5 years of tamoxifen versus tamoxifen alone for 5 years for postmenopausal women with node-negative disease. To evaluate treatment effect heterogeneity, we performed subgroup analyses of young versus old. The older cohort appeared to possibly benefit from CMF when patient age was dichotomized into two groups with <65 versus ≥65 years, but the younger cohort appeared to benefit when age was dichotomized using <60 versus ≥60 (see Fig. 79.2). Thus, the selection of cut points may influence the result of the subgroup analysis.

(c). Combination of characteristics—Subgroups can also be defined using combinations of characteristics. For example, the four widely measured immunohistochemical (IHC) biomarkers (estrogen receptor, progesterone receptor, Ki-67, and HER2) can be combined into a single continuous score known as the IHC4 score [29]. The development of such a score is usually based on a regression model from a particular study cohort, which is then validated using independent studies. The resulting score can then be used to define subgroups. Thus, it simplifies the problem of defining cut points for multiple characteristics to just one. However, the clinical interpretation of the subgroups defined based on the score could be diffi-

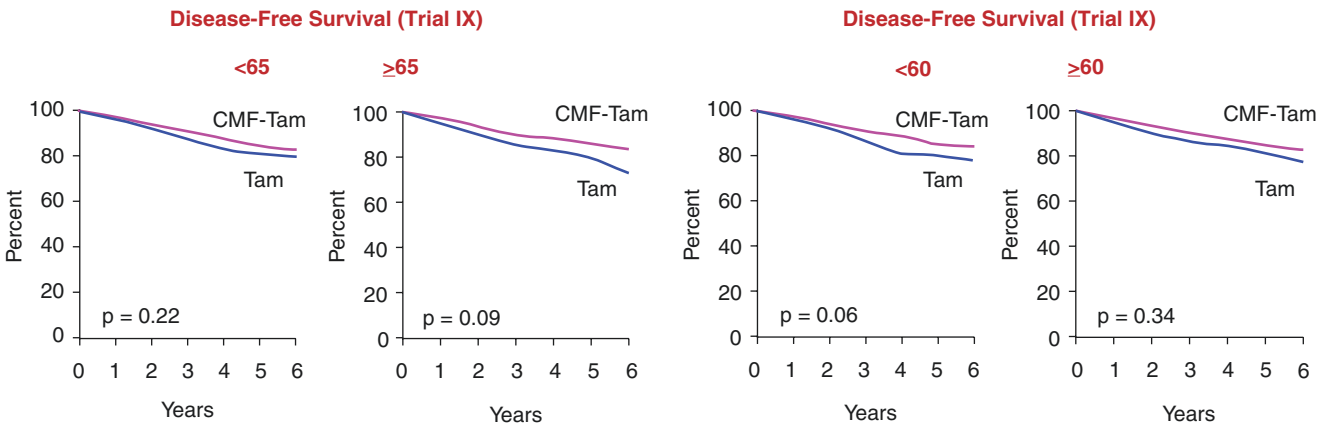

Fig. 79.2 The Kaplan-Meier plots on the *left* did not indicate any strong significance of the two age subgroups when the cutoff is at age 65 but may suggest the presence of an effect for the older group. The Kaplan-Meier plots on the *right* showed borderline-significant difference for the younger age subgroup when the cutoff is at age 60 (Courtesy Prof. R. Gelber)

cult. On the other hand, the definition of subgroups by stratifying with respect to a combination of characteristics is likely to produce a large number of subgroups with too few patients in them to perform useful analyses.

79.5.2 Guarantee-Time Bias (GTB)

Guarantee-time bias (also known as immortal time bias) occurs whenever an analysis that is timed from enrollment or random assignment, such as disease-free or overall survival, is performed to compare subgroups defined by a classifying event that occurs sometime beyond baseline during follow-up [30]. It is not trivial to recognize the potential for GTB, and even experienced investigators and journal editors can overlook the problem when a subgroup analysis is performed. For example, the NSABP B-30 trial, a randomized clinical trial, compared the effectiveness of concurrent versus sequential regimens of anthracyclines and taxanes. An unexpected finding reported that "chemotherapy-induced" amenorrhea appeared to be associated with increased survival among both ER-positive and ER-negative subgroups. Ovarian suppression has value in the treatment of breast cancer, but the effect was logically thought to be restricted to women with ER-positive disease [31]. The subgroup analysis, however, was biased because 24 months of follow-up was required to classify women in the amenorrhea subgroup, and those who relapsed or died before reaching 24 months (predominantly in the ER-negative group) were more likely to be classified as no amenorrhea. A subsequent corrected analysis of NSABP B-30 found no effect of amenorrhea for the ER-negative cohort and concluded that "women in whom amenorrhea developed as a consequence of adjuvant therapy had significantly better overall survival and disease-free survival than did women without amenorrhea, particularly when the tumor was ER-positive" [32]. Using data from the IBCSG 13-93 trial, the problem can be illustrated in the comparison of disease-free survival (DFS) according to amenorrhea status using naïve and 18-month landmark analyses. The naïve analyses, which suffer from GTB, showed highly significant reductions in hazards of DFS events independent of ER status, while the 18-month landmark analyses, which account for GTB, showed significant reductions only in women with ER-positive disease but not in women with ER-negative disease [30] (see Fig. 79.3).

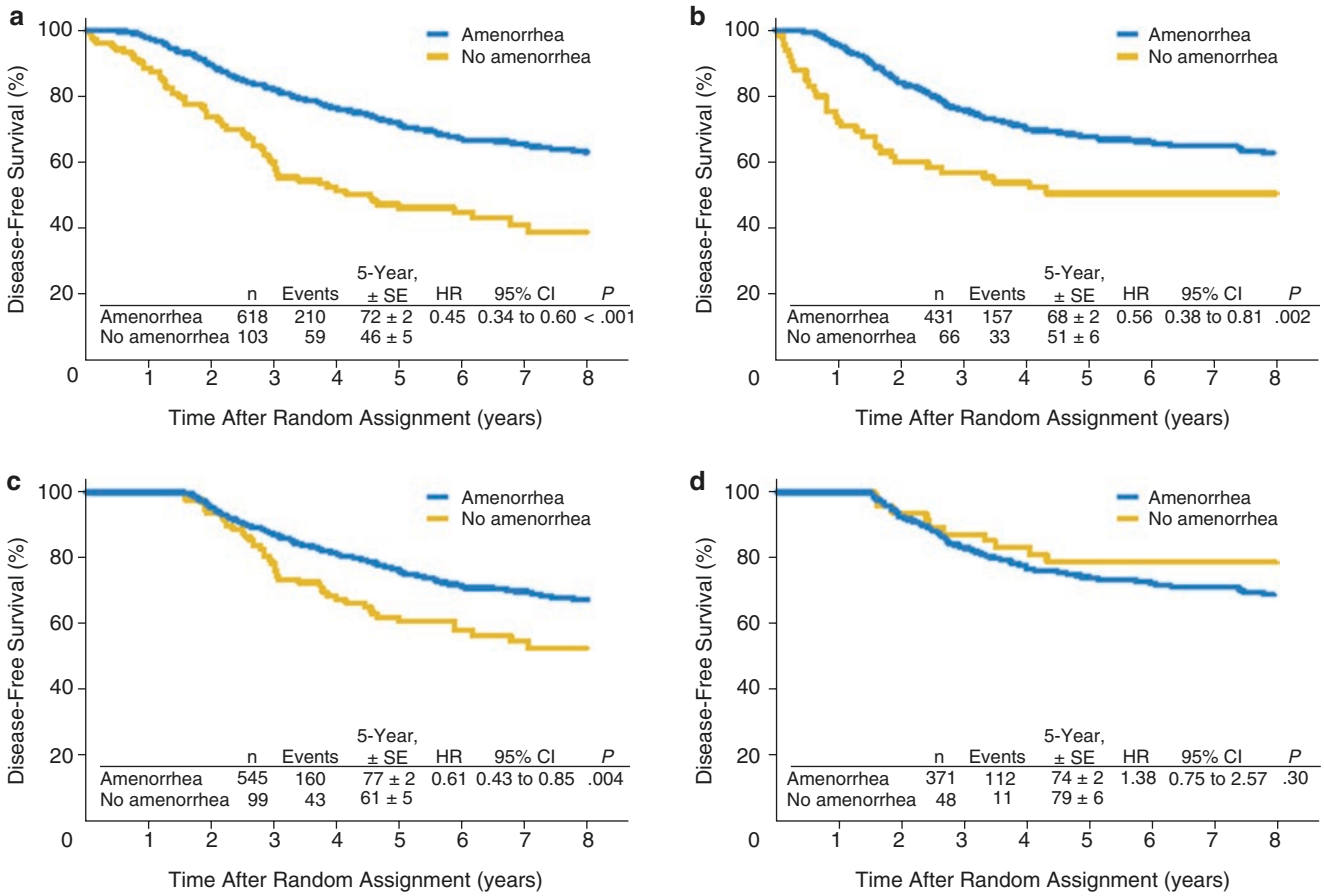

Fig. 79.3 Comparisons of DFS in IBCSG 13-93 according to amenorrhea status using naïve and 18-month landmark analysis. (**a**) Evaluates women with ER-positive disease and (**b**) with ER-negative disease using naïve analyses. (**c** and **d**) Evaluates the same subgroups using 18-month landmark analysis. The significant reductions in DFS of women with ER-negative disease disappear [25]. Reprinted with permission. © (2013) American Society of Clinical Oncology. All rights reserved

As described by Giobbie-Hurder et al. [30], three analytic techniques can be used to adjust for guarantee-time bias: conditional landmark analysis, extended Cox model with time-varying covariates, and inverse probability weighting.

79.5.3 Confirmation, Confirmation!

Many of the problematic issues can be resolved through confirmation. Once an effect in a subgroup is detected, confirmation can be obtained by looking at comparable subgroups in other clinical trials to see if a similar effect exists in the confirmatory datasets as was observed in the initial subgroup analysis dataset. Stronger evidence is obtained if several trials produce consistent results. For example, an evaluation of 2233 premenopausal patients who received chemotherapy alone without hormonal therapy in several IBCSG trials conducted prior to 1993 showed that those who were <35 years old with ER-positive disease had a particularly poor prognosis (*orange circles* in Fig. 79.4). This observation was contrary to expectations at the time as it was assumed that ER-positive disease was lower risk than ER negative. The unexpected IBCSG subgroup analysis required confirmation, which was obtained when NSABP, ECOG, and SWOG repeated the analysis on the premenopausal women who had received chemotherapy alone without endocrine therapy in their adjuvant trials [33, 34]. The estimates of the hazard ratios for the four subgroups (ER−, <35; ER+, <35; ER−, 35+; ER+, 35+ [reference group]) are plotted and show very similar outcomes (see Fig. 79.4).

Another example of the value of confirmation relates to the controversial role of bisphosphonates in early breast cancer. The AZURE [35] trial is a randomized clinical trial evaluating whether treatment with zoledronic acid, in addition to standard adjuvant therapy, improves disease outcomes in early-stage breast cancer. The protocol-defined secondary end points included analyses of effects in specific patient subsets and demonstrated that baseline menopausal status was the only factor that significantly influenced the effect of zoledronic acid on DFS. Results from the ABCSG-12 trial and the three related studies of Z-FAST, ZO-FAST, and E-ZO-FAST trials also support the proposition that zoledronic acid may be most effective for improving DFS in the adjuvant breast cancer setting for postmenopausal women or for those women with endocrine therapy-induced menopause [36]. Recently, a systematic review and meta-analyses of 13 trials came to a similar conclusion [37].

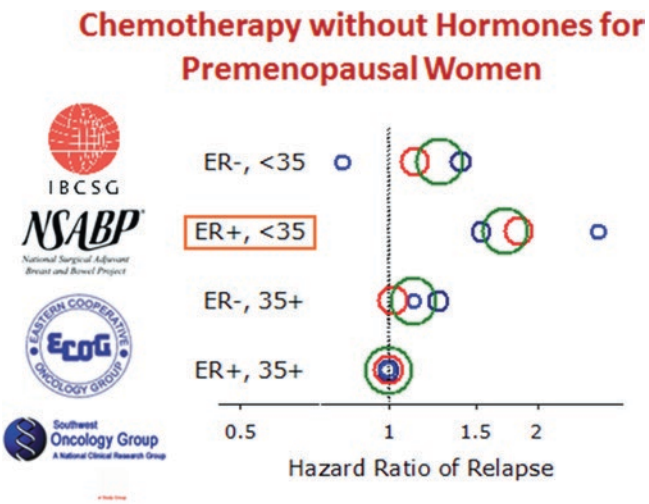

Fig. 79.4 The hazard ratios of relapse of four subgroups from four different clinical trials are plotted together. The results from IBCSG are represented by an *orange circle*, NSABP by a *black circle*, ECOG by a *blue circle,* and the Southwest Oncology Group by a *green circle* (Courtesy of Prof. Stefan Aebi)

79.5.4 Detection and Interpretation of Treatment Effect Heterogeneity Depends on the Scale Selected for Assessing Heterogeneity (Relative Differences or Absolute Differences)

Evaluation of treatment effect heterogeneity should be done in both absolute (e.g., absolute difference between two survival curves at a particular time point) and relative terms (e.g., hazard ratio). Absolute effects are useful for clinical purposes when individual treatment decisions are made between two competing therapies, which require assessment of benefit and risk for the patient. Relative effects are most important for comparing treatment effectiveness relative to a control group in the general patient population. An interaction detected between a covariate and treatment effect measured on the absolute scale may not be detected if the treatment effect is measured on the relative scale. For example, in the BIG 1-98, we evaluated whether Ki-67 labeling index was associated with treatment effect heterogeneity when comparing tamoxifen versus letrozole using the STEPP method described in the next section (see Fig. 79.5). If the treatment effect is measured in the absolute scale of the difference in 4-year DFS, we see a significant difference when Ki-67 is high, because both the predictive and the prognostic features of the marker influence the absolute difference (Fig. 79.5a, b). However, if the treatment effect is measured in the relative scale (hazard ratio), the relative effect is not significant (Fig. 79.5c) [11]. Thus, the significance of a test of heterogeneity in treatment effect may depend on the scale in which the effect is measured. Figure 79.5 will be explained in more details later on in the Sect. 79.5.5.2.

79.6 Tools for Subgroup Analysis

Given all of these issues, new methodologies and software have been developed to assist statisticians in performing subgroup analyses. These methodologies do not solve all of the issues, but they provide a set of tools that allow exploration. The following are some that are currently used.

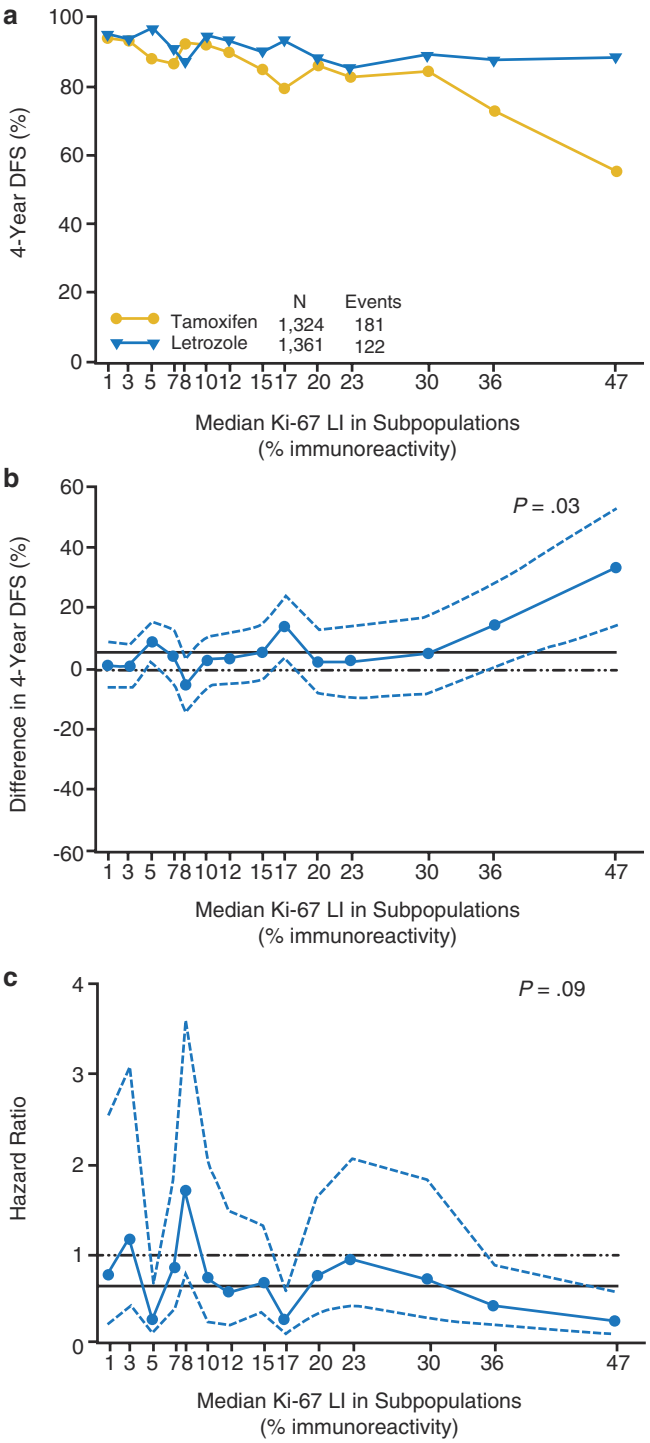

Fig. 79.5 Evaluation of treatment effect heterogeneity using biomarker Ki-67 (BIG 1-98) considering (**a**) 4-year DFS, (**b**) difference in 4-year DFS and (**c**) hazard ratio: using biomarker KI-67 in the BIG (Breast International Group) 1-98 RCT comparing letrozole with tamoxifen as adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer. STEPP analyses showed patients with higher Ki-67 values who were assigned to receive tamoxifen had the poorest prognosis and may benefit most from letrozole. [11, 38] Reprinted with permission.

79.6.1 Regression Model with an Interaction Term

The simplest approach is to use "standard" methodologies. As illustrated in the HERA trial subgroup analysis, one can use Cox proportional hazards model with interaction terms for the treatment effect by subgroups formed by each covariate. Thus, testing for any subgroup treatment effect is the same as testing for the null hypothesis that the coefficient for the interaction term in the Cox model is equal to zero. If the null hypothesis is rejected, one can conclude that there is evidence to support the hypothesis that the treatment effects in some subgroups may be different. It is important to remember to do multiple test corrections if more than one subgroup analysis is performed. The simplest is the Bonferroni correction which adjusts the significance level by the number of subgroup analyses done. Other advanced statistical methods such as false discovery rate (FDR) [39] can also be used. The subgroup analysis results are usually presented as a forest plot as shown in Fig. 79.1, with the solid vertical line drawn at the overall treatment effect [5], the fulcrum for interpreting treatment effect heterogeneity, as described earlier.

79.6.2 STEPP Analysis [11, 40–44]

STEPP stands for Subpopulation Treatment Effect Pattern Plot. It is a nonparametric tool to analyze survival data (Kaplan-Meier at a specific time point, cumulative incidence, or Cox proportional hazard models). In addition to survival data, it can be used to analyze continuous, binary, and count data that are modeled with generalized linear models. The key feature of STEPP is that one can create overlapping windows of subgroup populations based on a continuous covariate of interest. There are two methods used to define the overlapping subpopulation: sliding window and tail oriented.

A sliding window approach is the process of creating subgroups based on the continuous value of the covariate of interest, moving the windows from the lowest values of the covariate on the left and the highest values of the covariate on the right. The construction of the windows relies on two quantities: r1, minimum number of patients included in both adjacent overlapping subpopulations, and r2, minimum number of patients in each subpopulation. Assuming no ties, the first window consists of r2 patients with the lowest values of the covariate. Then, the next window consists of r2 patients but with a maximum of r2–r1 of the patients with the lowest values of the covariate replaced by r2–r1 of the patients with the next highest values. So, the first and the second windows will have r1 patients in common. Subsequent windows are created similarly from left to right by replacing r2–r1 patients

until the population runs out. The sliding window approach is useful for detecting treatment effect patterns along the continuum of covariate values. An example of how the subpopulations are constructed for a population of 749 patients along their ages ranging from 29 through 78 years is shown in Table 79.1. The minimum size of patients in each subpopulation is 100 (r2), and 70 (r1) patients are included in both adjacent overlapping subpopulations. Fifteen overlapping windows of subpopulations are created as a result. Note that due to ties, the sample size for each subpopulation is ≥100, and the sample size for the last subpopulation is less than 100.

One can also create a nested set of tail-oriented windows where nested subsets of windows are created starting with the entire population and creating subpopulations with progressively higher or lower median values for the covariate of interest. With this approach, the overall study population is used to obtain the treatment comparison result in the center of the STEPP plot. Subsequent windows to the left of center are constructed by successively removing patients with the highest covariate values, thus forming subpopulations with lower and lower median covariate values. Similarly, by successively removing patients with the lowest covariate values, windows to the right of center include patients with progressively higher values of the covariate. This method is intended to illustrate monotonic treatment effect patterns as the covariate value either decreases (to the left) or increases (to the right), and effects can be appreciated compared to the overall population result illustrated in the middle of the plot. The treatment effect is computed based on each subpopulation of each treatment group. Figure 79.6 illustrates the two subpopulation patterns.

Table 79.1 Subpopulation summary information of the 15 subpopulations constructed using a sliding window approach according to the age of patients

Subpopulation	Median Age (yrs)	Minimum (yrs)	Maximum (yrs)	Sample Size
1	43	29	47	112
2	46	42	49	100
3	49	46	50	100
4	50	48	51	101
5	51	50	52	106
6	52	51	54	121
7	54	53	56	107
8	57	55	59	128
9	60	58	61	108
10	61	60	63	106
11	64	62	66	107
12	66	64	68	102
13	68	66	71	105
14	70	68	74	103
15	73	70	78	92

The minimum number of patients per subpopulation (r2) is set to 100, and the largest number of patients in common among consecutive subpopulation (r1) is set to 70

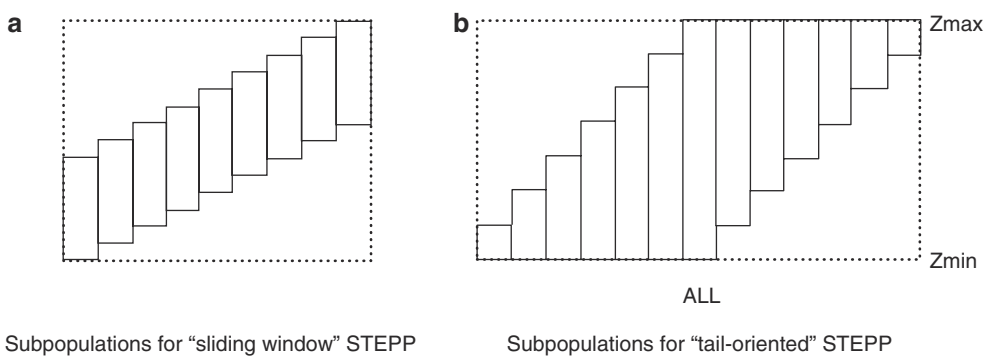

Fig. 79.6 Illustration of the two subpopulation patterns. Zmax and Zmin are the maximum and minimum values of the covariate of interest [41]. Plot (**a**) displays the sliding window and plot (**b**) displays the tail-oriented window. Reprinted from Biostatistics, Bonetti M, Gelber RD, Patterns of treatment effects in subsets of patients in clinical trials. 2004 5:465–481. © (2004) by permission of Biostatistics, Oxford University Press. All rights reserved

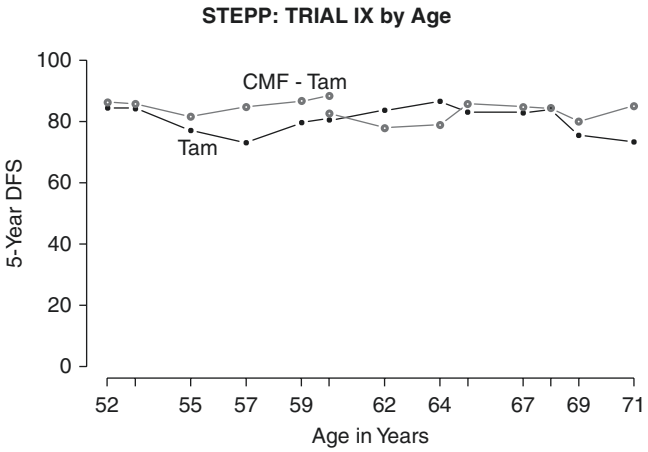

Fig. 79.7 The 5-year DFS outcome comparing CMF-Tam versus Tam alone along overlapping age subpopulations (Courtesy Prof. R. Gelber)

A STEPP plot shows the treatment effect estimates (or the *y*-axis) computed within the subpopulations.

For example, using the data from the IBCSG Trial IX example for postmenopausal, node-negative patients presented in Fig. 79.2, we performed a sliding-window STEPP analysis by creating subpopulations based on age. STEPP shows the pattern of 5-year DFS treatment effect across overlapping different age subgroups in one plot. As shown in Fig. 79.7, the CMF-Tam arm has slightly higher 5-year DFS compared with Tam estimated for patients under 60, but estimates cross between ages 60 and 65 and then again are higher than Tam alone for the oldest age group. Thus, there is no clear pattern that age is a covariate modifying the magnitude of treatment effect in this trial. By contrast, if we examine the sliding-window STEPP analysis for this trial exploring the relationship of CMF-Tam versus Tam alone according to level of ER expression for postmenopausal women with node-negative breast cancer, we see a clear relationship between the benefit of three courses of CMF prior to tamoxifen for the patients with the lowest levels of ER expression, in Figs. 79.8a and 79.8b. This benefit rapidly diminishes as the values of ER expression reach and exceed 10 fmol/mg cytosol protein. The pattern highlighted in the STEPP analysis is reflected in the Kaplan-Meier plots shown in Fig. 79.9 illustrating the treatment differences separately for ER-negative (<10 fmol/mg) and ER-positive (≥10 fmol/mg) subgroups.

The choice for minimum size of overlapping subpopulation (r1) and minimum subpopulation size (r2) is application specific. In order to get a good estimate, one should pick a large r2 value; if a smoother plot is desired, one should pick a larger r1 value. One may need to try a few combinations of r1 and r2 to obtain the desired STEPP plots.

A complete survival STEPP analysis provides three separate plots allowing us to see the treatment effect patterns in the two treatment arms separately, as well as evaluating the heterogeneity in treatment effect differences on an absolute and

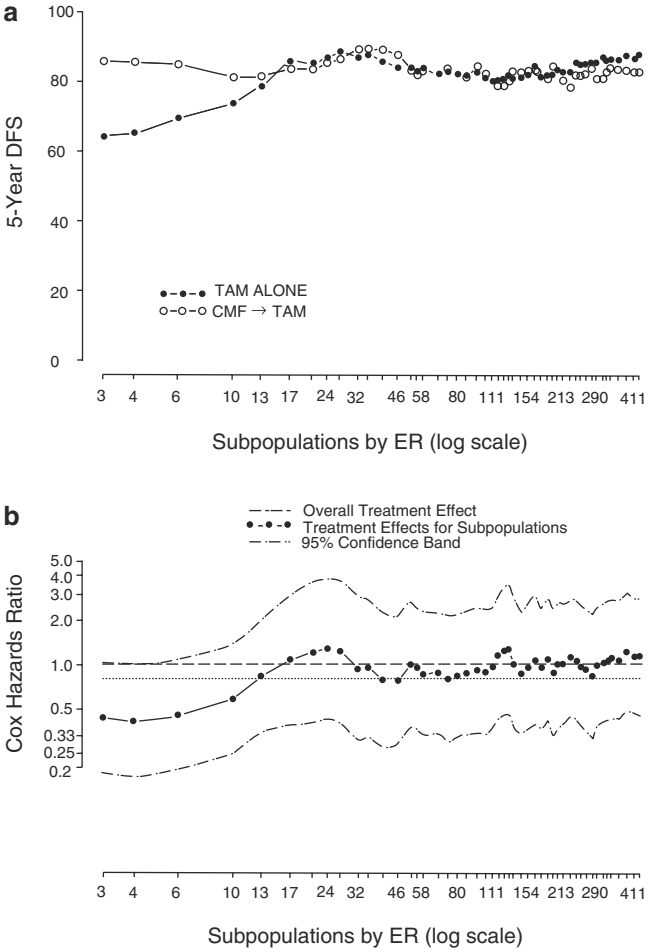

Fig. 79.8 STEPP analysis comparing CMF-Tam versus Tam alone according to estrogen receptor (ER) value in IBCSG Trial IX. It shows a much larger treatment effect for patients with low (ER-) disease. For this sliding-window analysis, each subpopulation contained approximately 200 patients, and each subsequent subpopulation was formed moving from left to right by dropping ten patients with lowest values of ER from the subpopulation and adding ten patients with the next higher values of ER. Plot (**a**) displays the absolute treatment effects in terms of 5-year DFS, while plot (**b**) displays the Cox model hazards ratio (HR) according to values for quantitative ER [28]. Reprinted from JNCI, IBCSG, Endocrine responsiveness and tailoring adjuvant therapy for postmenopausal lymph node-negative breast cancer 2002; (94) 14:1054–1065. © (2002) by permission of JNCI, Oxford University Press

on a relative scale. As an example, we evaluated the treatment effect heterogeneity using biomarker Ki-67 data from the BIG 1-98 trial [39, 45]. The three plots are shown in Fig. 79.5.

1. A treatment effect plot displays the treatment outcome of each therapy against the median of the covariate value for each subpopulation. One can visually discern if there is emerging treatment heterogeneity between the two treatment groups Fig. 79.5a.
2. A difference plot displays the absolute difference of treatment effects along the subgroups defined by the covariate Ki-67 Fig. 79.5b.

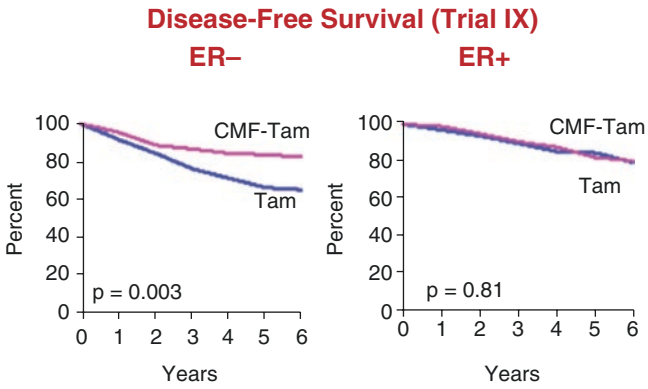

Fig. 79.9 The Kaplan-Meier comparison of the two treatment arms of CMF-Tam versus Tam on subgroups of patients with ER– and ER+ diseases in IBCSG Trial IX (Courtesy Prof. R. Gelber)

3. A relative difference plot displays the relative difference of treatment effects along the subgroups defined by Ki-67. In the survival setting, the treatment effects would be measured as hazard ratios Fig. 79.5c.

In order to assess statistical significance of the differences, STEPP performs a test by permuting the covariate values across patients in each subgroup within each treatment under the null of no heterogeneity of treatment effects. The supremum statistic which is the absolute deviation from the overall effect is computed for each permuted sample. The permutation supremum *p*-value is the percentage of results which are more extreme than the observation and is presented together with these plots so that one can meaningfully interpret the results. A significant result can imply that the observed pattern of treatment effects may not be due to sampling variation.

In large randomized controlled trials, the risk of random imbalance of the covariates is mostly negligible. However, with STEPP subgroup analysis in smaller studies, the imbalance may be substantial. To alleviate this problem, one can choose a larger minimum subpopulation size (r2) with a bigger overlap (r1). Adjustment for known confounders using regression can also reduce a biased assessment of the treatment comparison in GLM models with STEPP.

STEPP offers several advantages compared to other statistical approaches. It does not require predefinition of specific cutoff points for developing patient subgroups. Treatment effect heterogeneity is illustrated graphically, allowing for a convenient exploratory evaluation. It does not rely on the assumptions of a regression model. It provides an overall *p*-value for testing whether treatment effect heterogeneity is significant. It can be based on absolute or relative treatment effects. Also, treatment effect is defined through traditional measures computed on well-defined patient subgroups. Lastly, STEPP can be applied in various survival data analysis contexts such as Kaplan-Meier, Cox proportional hazards, cumulative incidence, and generalized linear models. A free R [45] software package, **stepp**, is available for download through CRAN. The current version of the software provides support for comparison of two treatments with the sliding window approach. The following standard models can be used with the software: Kaplan-Meier, Cox proportional hazards, cumulative incidence, and generalized linear models (Gaussian, binomial, and Poisson) with their canonical links.

STEPP analysis also has some drawbacks. It allows only one covariate of interest to be analyzed at a time, although several covariates can be combined into one composite risk score. STEPP cannot be used to identify a definitive cutoff point for clinical uses but rather illustrates patterns of treatment effect. A separate confirmatory trial or application of other methods is needed to identify the appropriate clinical cutoff point.

79.6.3 Fractional Polynomial [46]

A different approach to modeling interactions between treatment and continuous covariates in clinical trials has been proposed by Royston and Sauerbrei. Fractional polynomial (FP) is a flexible technique and has been used successfully in developing many models. The main idea is to use FP modeling of outcome and testing equality of regression coefficients between treatment groups in an interaction model adjusted for other covariates. The authors' extensive experience with this method suggests that a two-term fractional polynomial (FP2) function may describe the effect of a prognostic factor on a survival outcome quite well. They also recommend checking the results by plotting Kaplan-Meier survival curves and by estimates of treatment effects in subgroups.

Importantly, they also provide a software package, MFPI, in Stata, that can also be used to explore the heterogeneity of treatment effects along a continuous covariate.

79.6.4 Bayesian Models and Other Sophisticated Techniques

There are other subset analysis methodologies developed based on Bayesian principles. One such approach is described by Simon [47] and illustrated using a Cox proportional hazards model with independent and normal priors. The computations required for using this approach are straightforward and require no specialized software.

Some more theoretical approaches are also available. A local partial likelihood estimation (LPLE) technique can be used to estimate nonlinear interactions under a proportional hazards model [48]. A two-stage estimation procedure for subject-level treatment differences for a future patient's disease management and treatment selections is also available [49].

The few methods that we listed here are by no means exhaustive. As a general suggestion, investigators are encouraged to use multiple methods when doing subgroup/subset analysis. Consistent evidence of heterogeneous treatment effects would be reassuring. If there is a lack of consistency, understanding the differences may help to explain the underlying heterogeneity and help to identify important subgroups.

Conclusion

Subgroup analysis is a general statistical methodology, which is applicable to clinical research. It follows the targeted therapy principle—if the treatment effect varies across subgroups, then therapies can be prescribed with the most optimal therapeutic benefit for the individual patient. This would both improve patient disease outcomes and reduce harm, as well as costs, in many clinical situations. However, it is imperative that analyses are performed properly. Otherwise, they may misguide research or harm patients.

Simply abandoning subgroup analyses because of past mistakes is not acceptable. In fact, avoiding subgroups is a very steep price to pay [21]. We must have strict guidelines for conduct—be skeptical at all times, propose subgroup analyses in the protocol document, do exploratory analyses based on a variety of prespecified heterogeneous factors, and never perform "data dredging." If promising results are detected, they need to be replicated in independent series because subgroup analyses are subject to large statistical variation. Ideally, a properly designed trial with sufficient power should be conducted to confirm the result. In short, analyses must be predefined, carefully justified, and limited to a few clinically important questions, and post hoc observations should be treated with skepticism irrespective of their statistical significance. Stringent guidelines for reporting must also be followed [7].

Despite various pitfalls, many subgroup analysis methodologies have been proposed, and software have been developed in the past decade which help researchers identify proper subgroups and facilitate their analyses. STEPP, MFPI, and Bayesian approaches can now be used to investigate heterogeneous treatment effects, and some of these approaches have freely accessed software packages available for download. Unfortunately, there are still only few published papers exploring heterogeneity in breast cancer clinical trials deploying these new techniques. As increasing amounts of genomic and biomarker information becomes available in clinical studies, subgroup analyses may help bring great benefit to all cancer patients by contributing to the development of personalized medicine.

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The Methodology of Meta-Analyses and Its Potential Contribution to Patient Care

80

di Sara Gandini

80.1 Introduction

The art of meta-analysis, the combination of results from multiple independent studies, is now more than a century old. In the last 40–50 years the impact of meta-analysis has grown enormously. The increasing rate of publications of meta-analyses is exponential and by far surpasses the increase in the rate of publications of RCTs [1]. This rapid increase in the number of published meta-analyses is mainly due to a greater emphasis on evidence-based medicine and the need for reliable summaries of the vast and expanding volume of clinical research. A systematic review of the relevant external evidence provides a framework for the integration of the research, and meta-analysis offers a quantitative summary of the results, necessary for evidence-based medicine.

Meta-analysis is a process that includes results of independent studies as well as a quantifiable combination of results estimates [2]. It provides a systematic approach to selecting and integrating findings across studies and to control for chance and potential bias. It is a methodology used for contrasting and combining results of different studies, where the individual unit of the statistical analysis is the study. This approach allows hypothesis testing regarding sources of heterogeneity and quantification of biases. Meta-analysis can also help to identify gaps in knowledge found in the published literature and thus can help provide guidance for future research.

Meta-analysis differs from qualitative or narrative review because conclusions from publications are not only discussed qualitatively but also involve a quantitative assessment of the available published data. Narrative reviews may present several problems because they do not have tools to analyse and quantify results of single studies and they are easily influenced by several biases (e.g. publication bias and reviewer bias). In fact without the obligation to clearly state inclusion criteria, it is likely that researchers include studies that support their own opinion and ignore those that do not. Cooper and Rosenthal [3] showed that even with only seven studies, narrative and quantitative reviews led to different results. However in a research area with very few studies often a narrative and critical review of the studies are perhaps the more suitable approach.

Pooled analyses are special types of meta-analyses that include individual subject data obtained from authors of the papers, and it is characterized by numerous advantages. It allows analyses among exposures and confounders, not investigated in the original studies, and it required variables to be recoded across studies to make them more compatible and to make adjustments to deal more extensively with heterogeneity. A major impediment to this kind of meta-analysis is the fact that it is very time-consuming because it requires several years just to obtain the data and demands close cooperation between the authors of the studies. Therefore meta-analysis of published data is often considered a sound approach when resources and time are limited and when original study data are not available.

The Cochrane Collaboration, launched in 1993, has been influential in the promotion of evidence-based medicine. This international network of individuals is committed to preparing, maintaining and disseminating systematic reviews of research on the effects of healthcare. Their reviews are made available electronically in the Cochrane Database of Systematic Reviews, part of the Cochrane Library (http://www.update-software.com/cochrane).

Meta-analysis techniques provide a useful means of summarizing overall efficacy results of clinical trials and analysing less frequent outcomes in the overall safety evaluation. Meta-analysis also has a useful role to play in the generation of hypotheses for future studies.

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80.2 Meta-analysis of Observational Studies

The need to assess risks that are small, but that may have large public interest or have important implications for public health, has amplified their use in summarizing the evidence. However the use of meta-analysis for published observational studies is less accepted than in the area of clinical trials for their intrinsic biases and differences in study designs. The most prominent arguments against meta-analyses are the fundamental issues of confounding, selection bias, as well as the large variety and heterogeneity of study designs and data collection procedures in epidemiological research. Despite these controversies, results from meta-analyses are often cited and used for decisions. They are often seen as the fundamentals for risk assessment. They are also performed to summarize the current state of knowledge often prior to designing new studies.

Conflicting results among studies may arise when sample sizes of individual studies are too small to find stable results. Actually most epidemiological studies are too small to detect anything but a comparatively large relative risk (RR) associated with a fairly common exposure. Thus meta-analyses may become a useful tool to evaluate weak risk factors that have large public health impact. An increase of risk of only 20% of certain cancers, for example, may involve millions of people, and to detect such small increases in risk, huge studies are necessary [4].

An important function of meta-analysis is the investigation of between-study heterogeneity that is an opportunity to understand study variation. Meta-analysis can lead to insights when study design, exposure assessment or exposure levels, study populations, etc. are found to relate to study outcome. Thus investigation of heterogeneity can provide interesting hypotheses for future analyses and should be viewed as strength of meta-analysis, not a barrier to its use. Actually if all of the studies show same results, meta-analysis would not be very useful because it would not provide much more information than the original studies. Furthermore between-study heterogeneity was very often found significant, even when the authors considered subsets of studies identified with strict inclusion criteria. In fact many analysts identify heterogeneity and deal with it by excluding studies until a satisfactory degree of homogeneity is achieved. Authors sometimes exclude 25% of the data and still generalize to the total population.

A systematic revision of all the literature for a comprehensive meta-analysis allowed an in-depth exploration of associations and interactions among risk factors and provided some clues towards the epidemiology of a disease by looking extensively at inconsistencies and variability in the estimates. A good meta-analysis should help to understand differences in results from the mass of papers from which they are derived.

Meta-analysis permitted questions to be debated on whether the association of a disease with risk factors may depend on the composition of the population under study, the level of exposure in the study population, the definition of disease employed in the studies or the methodological quality of the studies.

80.3 Pooled Analyses

Meta-analyses of individual patient data can play a useful role in providing evidence for future studies. However, they can be very challenging from a statistical perspective in terms of dealing with the problems that plague meta-analyses in general.

Mostly very simple methods are used, such as standard fixed effect meta-analysis model for aggregate data [5]. Sometimes within-study comparisons have been adjusted for confounding variables before application of traditional meta-analytic techniques for summary data. Alternatively, more complicated regression techniques have been applied to produce stratified estimates of a common estimate adjusting for covariates.

When a two-stage approach is adopted, the first stage involves reanalysing each study using the model appropriate for the design with study-specific confounders. The second stage then combines the study-specific estimates using standard meta-analytic techniques. When the individual studies are large, two-stage methods produce nearly unbiased exposure estimates and standard errors of the exposure estimates from a generalized linear mixed model. By contrast, joint fixed effects logistic regression produces attenuated exposure estimates and underestimates the standard error when heterogeneity is present. While bias in the pooled regression coefficient increases with within-study heterogeneity for both models, it is much smaller using the two-stage model.

Random effects meta-analyses, meta-regression and assessment of the effects of patient-level covariates across all studies have been developed [6] but appear to be seldom used in practice. However heterogeneity between studies would exist, and a random effects model is usually to be more appropriate model than a fixed effect one.

Pooled reanalyses are mostly performed by combining data from studies of the same type only. Statistical issues of pooling data from case-control studies have been investigated by Stukel et al. [7]. An overview of methodological aspects for combining individual patient data with aggregate data, to utilize all the evidence available, is presented by Riley et al. [8].

The prospectively planned pooled meta-analyses include pooling as part of the protocol in order to standardize data collection procedures and definitions of variables for the individual studies. Joint planning of the data collection and analysis increase the homogeneity of the included data sets; however, in contrast to multicentre randomized clinical trials, important heterogeneity between the study centres still may exist. This heterogeneity may arise from differences in populations, in the relevant confounding variables (e.g. race may only be a confounder in some centres) and potentially differences in ascertainment of controls [8].

80.4 Statistical Methods

Like primary research, meta-analysis proceeds by framing a research question to be addressed, by sampling a defined population of completed primary studies to be surveyed, coded for relevant methodological characteristics and analysed to test hypotheses derived from the research question.

First of all, a good meta-analysis should begin with a systematic complete review of the literature. An effort should be made to obtain studies from all sources, literature databases and reviews, from published and unpublished literature, to attempt to minimize bias. After defining inclusion and exclusion criteria, a flow diagram should be provided to describe how the meta-analyst arrives the final group of studies included in the analysis (a blank version of the flowchart can be downloaded at http://www.consort-statement.org/QUOROM.pdf).

Afterwards, the meta-analyst builds a database extracting important information from each retrieved study. From each paper we need at least to obtain a risk estimate/effect size with a measure of its precision that can be obtained from standard deviation (SD), confidence intervals, *P*-values or crude data.

Lastly, findings from individual studies can be combined using appropriate statistical methods. The different methods use a similar approach in which the estimate from each study is weighted by the precision of the estimate through fixed or random effect models.

The usual way of displaying data from a meta-analysis is by a pictorial representation (known as a forest plot). This displays the findings from each individual study as a blob or square. The size of the blob or square is proportional to the weight (precision/statistical power) of the study estimate. A horizontal line (usually the 95% CI) is drawn around each of the studies' squares to represent the uncertainty of the estimate. The summary estimate obtained by combining all the studies is usually displayed as a diamond.

Guidelines for reporting Meta-analysis Of Observational Studies in Epidemiology (MOOSE) have been published by Stroup et al. [9, 10]. Especially the detailed description of methods is required so that the analysis could be replicated by others. The proposed checklist contains specifications for reporting background, search strategy, methods, results, discussion and conclusion. Use of the checklist should improve the usefulness of meta-analyses for authors, reviewers, editors, readers and decision-makers.

Many studies will use similar but not identical endpoints, and we have to translate them into some common measure: for continuous endpoints usually mean differences with standard deviations (SD), for binary endpoints usually relative risks or odd ratios with 95% confidence intervals (95% CI). Often the distinctions among the various measures of relative risk, such as RR, odds ratios (OR) and incidence rate ratio, are ignored, because disease under investigation is rare.

Omitting study results from the meta-analysis because a measure of the SD cannot be obtained should be avoided. Meta-analysts are forced to improvise and/or use other summary measures to derive an SD estimate to include studies with missing SDs. When SDs cannot be algebraically recalculated from reported data, meta-analysts have suggested and used a myriad of methods to impute SD (fill in SDs with plausible values) to attenuate any loss in power and to avoid bias. Wiebe et al. [11, 12] review several methods of imputation and suggested with Robertson et al. [12] to use multiple imputation in a variety of sensitivity analyses for handling missing SD in meta-analyses.

For survival data, when hazard ratios are not reported, a number of methods of estimating them are presented and discussed by Parmar et al. [13].

Fixed effects models are based on the mathematical assumption that a single common (or "fixed") effect underlies every study in the meta-analysis. In other words, if we were doing a meta-analysis of odds ratios, we would assume that every study is estimating the same odds ratio. Under this assumption, if all studies were infinitely large, they would produce identical results. This means that between-study heterogeneity is not statistically significant [14].

Random effects models make the assumption that individual studies are estimating different underlying risks. The idea of a random effects meta-analysis is to learn about the distribution of risks across different studies.

When large heterogeneity is found, diversities in the designs and analyses of the various studies should be taken into account in the final model, and it can be assumed that the true effects estimated will vary among studies. There are two sources of variability that must be addressed, the usual sampling variation in the estimates and variation in the underlying parameter. To account for both sources of variation in the meta-analysis, the DerSimonian and Laird [15] method is often used, especially for clinical trials.

Besides the moment-based method by DerSimonian and Laird method, summary estimates can be obtained using likelihood-based methods [16]. Estimates based on likelihood

methods offer the advantage that they provide the option to formally test which model is appropriate for the data by applying the likelihood ratio test.

The consequence of performing a random rather than a fixed effects model is that usually the confidence intervals for the summary estimate are wider. A random effect analysis therefore suggests more conservative in estimating the underlying parameter than a fixed effects model does. Fixed effects summaries are preferable to random effects models when studies are very similar in their methods, in their populations at risk, in their exposure contrasts and in their results. However estimates from random effects models tend to be more sensitive to publication bias than fixed effect estimates, because smaller studies have larger relative weights. It follows that random effects models will be more strongly biased than fixed effects models by any tendency not to publish small statistically non-significant studies.

Analysis of heterogeneity, especially analysis of study characteristics that might explain differences among the results, provides the most important information for interpretation and decision-making than can be provided by any single summary.

80.5 Treatment Difference

Many meta-analyses concern the comparison of two treatments in terms of a selected set of outcome measures. For each chosen outcome measure, the aim is usually to estimate and make inferences about the difference between the effects of the two treatments. This involves choosing an appropriate measure (parameterization) of the treatment difference and calculating individual study estimates and an overall estimate of this difference. A traditional meta-analysis is one in which the overall estimate of treatment difference is calculated from a weighted average of the individual study estimates.

Meta-analyses may be performed on studies for which the available data are in the form of summary information from trial reports or publications or on studies for which individual patient data are available. The form of the data available from each study has implications for the meta-analysis, and here three forms which are commonly encountered are considered.

The first consists of an estimate of the treatment difference and its variance or standard error—the minimum amount of information needed. If a study provides an estimate of treatment difference which is not an estimate of the chosen parameterization, it may not be possible to include it. For example, in the context of binary data, we may wish to estimate the log-odds ratio, and so a study for which only an estimate of the probability difference is available cannot be used.

The second form of data is slightly more detailed, consisting of summary statistics for each treatment group, enabling a choice to be made between several different parameterizations of the treatment difference. For example, in the context of normally distributed data, knowing the sample size, mean and standard deviation for each treatment group allows estimation of the absolute mean difference or the standardized mean difference.

In an individual clinical trial the likelihood ratio test is frequently used to test the hypothesis concerning the treatment difference. The maximum likelihood (ML) estimate of the treatment difference is then typically presented with a standard error or confidence interval. ML estimation has the advantages of asymptotic optimality and general availability in statistical packages. This is the principal method of estimation which is presented in this book. As ML estimation involves iterative procedures and is usually performed via a statistical package, a specification of the methodology is presented together with a SAS procedure which could be utilized. The likelihood approach to a single clinical trial can be extended to the meta-analysis of all of the trials when individual patient data are available.

A simpler approach to estimation, based on the efficient score and Fisher's information statistics, has been widely used for meta-analysis. This approach, on which a number of commonly used statistical tests are based, produces approximate ML estimates.

80.6 Meta-analysis Dilemma: Heterogeneity

Meta-analysis should not be applied merely as a statistical method, which combined published results, because nonexperimental studies do not allow for the assumption that the variation between studies is only attributable to statistical sampling error. It is unlikely that this so-called homogeneity assumption is fulfilled. Part of the variation in the estimates is due to differences in definitions, in measurements of the exposure, features of the studies and of the populations. A systematic investigation of between-study heterogeneity, as a function of differences in design features, types of analyses and population characteristics, helps to explain the controversy between study results and provides interesting considerations for cancer epidemiology.

An important function of meta-analyses is the exploration of sources of variation in study results, which should be viewed as strength of this work. Investigation of biases and inconsistencies should become one of the key phases for a meta-analyst because it can lead to more insights than the mechanistic calculation of an overall measure of effect, which will be often biased. Exploration of sources of heterogeneity can lead to insights over modification of apparent associations by various aspects of study design, exposure measure and population and may allow identification of features of study design that may have implications for future research.

There may be different kinds of heterogeneity: population heterogeneity and methodological heterogeneity. The term "population heterogeneity" covers factors such as study location, age, sex and types of diseases. Methodological heterogeneity has to do with differences between study designs and analyses. When different studies give different results, the aim of the meta-analysis should be to investigate the reasons why effects differ across studies, identifying methodological discrepancies among studies, assessing the possibility of confounding factors and evaluating differences among populations under study [17, 18].

Subgroup analyses and "meta-regression" are techniques useful to work out when particular characteristics of studies are related to the sizes of the estimates. Subgroup analyses are meta-analyses on subgroups of the studies that partition the observed effect size variability into two components: the portion attributable to subject-level sampling error and the portion attributable to other between-study differences. This is obtained dividing results into different types of subjects, outcomes or study characteristics, but it requires cautious interpretation. When several outcomes are measured, but only a selected subset of them are reported and discussed, it is possible to have misleading results. Furthermore the more subgroup analyses are performed, the more likely it is that a statistically significant result will be found due simply to chance. Any subgroup analysis should be best considered as generating hypotheses for testing in the future and should have a scientific rationale [19].

"Meta-regression" models represent a useful tool to investigate possible explanations for between-study heterogeneity because they allow testing interactions between factors. The term meta-regression indicates the use of study-level covariates, as distinct from regression analyses that are possible when individual subjects data on outcomes and covariates are available. Subgroup analysis is equivalent to meta-regression with a categorical study-level covariate. Considering subgroup analysis formally as a meta-regression has advantages, since it properly focuses on the differences between subgroups, rather than the effects on each subgroup separately. Also random effects models allow for residual heterogeneity, not explained by subgrouping [19–21].

When there is a small number of studies and many differing characteristics, the risk of obtaining a spurious explanation from meta-regression is high. This is a particular problem in meta-analysis because there are many characteristics, which differ among the studies, and these can be highly correlated. Further summarizing of subject's characteristics at study level implies the risk of completely failing to detect genuine relationships between these characteristics and the size of risk factors. Meta-analysis carried out on individual subjects data can alleviate some of these problems. In particular within-study and between-study relationships can be more clearly distinguished, and confounding by individual level covariates can be investigated. However, as we have seen earlier, this kind of meta-analysis is much more expensive and time-consuming and very often not feasible [22].

Since poor-quality studies sometimes produce systematically different results, a meta-analysis may yield misleading results if the quality of the studies is poor [23]. In an attempt to cope with these problems and to control heterogeneity, many researchers restrict analysis considering some inclusion criteria. However an extensive investigation of the effect of inclusion criteria on results is recommended to avoid introduction of reviewer's own bias.

Use of quality scoring in meta-analysis is controversial because its validity is not clear and may not be associated with quality. It is very difficult to score and measure quality that is best evaluated qualitatively [24, 25]. Greenland called quality scores "perhaps the most insidious form of subjectivity masquerading as objectivity" because they modify data information by using arbitrary judgements in their assignment [26].

Sensitivity analysis is recommended rather than quality scores because it helps to establish the influence of individual study results to the overall summary estimate. The main role of a sensitivity analysis is to discuss robustness of results and determine whether the assumptions or decisions to either exclude or include studies have a major effect on the final estimates [27].

When working with observational studies, it is very likely that heterogeneity exists between the studies. After exploration of the contributions of all known or suspected factors that may have introduced variation in the estimates, heterogeneity remains very often unexplained. If appropriate statistical models are applied, then heterogeneous studies may still be pooled and statistical models that can take into account this variation, as random or mixed effects models, are essential.

Addressing statistical heterogeneity is one of the most troublesome aspects of many meta-analyses. The interpretative problems depend on how substantial the heterogeneity is, since this determines the extent to which it might influence the conclusions of the meta-analysis. It is therefore important to be able to quantify the extent of heterogeneity among a collection of studies. An obvious means of achieving this is by estimating the between-study variance of the parameters of interest. A common way of indicating the extent of heterogeneity is a statistical test (chi-squared test, i.e. Q test). A P-value is frequently quoted as an indication of the extent of between-study variability. The test has poor power with few studies and inappropriately high power with many studies, and it can therefore be difficult to decide either whether heterogeneity is present or whether it is clinically important. The test does not therefore provide a relevant summary of the extent to which heterogeneity impacts on the meta-analysis. In order to overcome the shortcomings of this

test, Higgins and Thompson [20] have proposed three indices for assessing heterogeneity in a meta-analysis. As they are interrelated, here we focus on the I^2 index, because of its easy interpretation. I^2 does not depend on the number of studies; therefore it is recommend to be presented together with the Q test. In fact this measure quantifies the impact rather than the extent of heterogeneity in a meta-analysis, and it can be compared across different effect metrics. I^2 describes the percentage of variability in point estimates that is due to heterogeneity rather than sampling error.

80.7 How to Deal with Sources Bias

One of the most crucial steps in a systematic meta-analysis is study identification. In order to control the biases, the process of identifying and selecting studies is very important. Refined methods to search for studies are fundamental to include all potentially eligible studies. The effect on the estimates of the exclusion of each study not contributing to the analysis should be investigated. In fact, if the studies included are a biased sample of all the studies conducted, then the force of any possible inference is limited.

Publication bias is the most mentioned bias in meta-analysis because when it is present the significance of results may influence whether a study is submitted, positively reviewed and eventually accepted for publication or not. Commonly meta-analysts refer to publication bias, but this is only one of the possible biases, included in the term "dissemination bias". In general when the analysis is influenced by the accessibility of research findings, we should talk of dissemination bias. This depends not only on whether a study is published but also on when, where and in which format this occurs [28]. For example, language bias is related to the fact that studies without significant results are preferably published in languages other than English, and this implies that it will be more difficult to find such "negative" studies. Authors try more likely to publish positive findings in an international, English language journal, whereas negative findings end in local journals. Therefore bias could be introduced in meta-analyses based exclusively on studies published in English [29]. Moreover if most of the major west European journals, published in languages other than English, are indexed in Embase or Medline, this is not the case for journals published in less-developed countries. Obviously it will be very difficult to find studies that are published in journals not indexed in one of the major databases.

Searches in computerized databases are usually extended examining the reference lists of other studies and reviews. When reference lists are used, citation bias may have an important role. Citation bias leads to underreporting of "negative" studies being referred to less often. On the other hand, significant results are sometimes published in more papers, increasing the probability for them to be discovered (multiple publication bias). Furthermore it is not always obvious that more publications come from a single study, and one data set may thus be included in an analysis twice.

Another source of bias comes from differences in methodological quality of studies. Methodological accuracy of smaller studies is not at the same level of larger studies, and papers of lower quality also tend to show larger effect estimates [23]. In these cases there is often an interaction between sample size and statistical significance. To publish a non-significant result, sample sizes must be very large. This is reasonable because the statistical power to detect a significant difference is low when samples are small. The real problem arises when the true relationship is modest as in the majority of epidemiological analyses [25]. In fact, for studies with small samples, the only results published will tend to be those that are significant, and this can lead to a systematic overestimation of the true effect size.

In meta-analysis, funnel plot and related statistical analyses are the most commonly used methods for assessing the possible existence of publication bias. Funnel plots are simple scatter plots of the risk estimates, on the *x* axis, versus some measure of their precision, as standard error, variance, inverse of variance and sample size, on the *y* axis. The name "funnel plot" arises from the fact that risk estimates from small studies will spread out more widely at the bottom of the graph, with the spread narrowing among larger studies that present more precise estimates (smaller standard errors). In the absence of bias, the plot should look like a symmetrical inverted funnel (see Fig. 80.1). If there is a bias, because, for example, smaller studies without statistically significant effects remain unpublished, this will lead to an asymmetrical form of the funnel plot with a gap in a bottom corner of the graph (see Fig. 80.2): smaller studies, with low power and greater standard errors, will not be published. The more the asymmetry is pronounced, the more likely it is that the amount of bias will be substantial and the pooled effect calculated from meta-analysis will probably overestimate the true risk estimate [29].

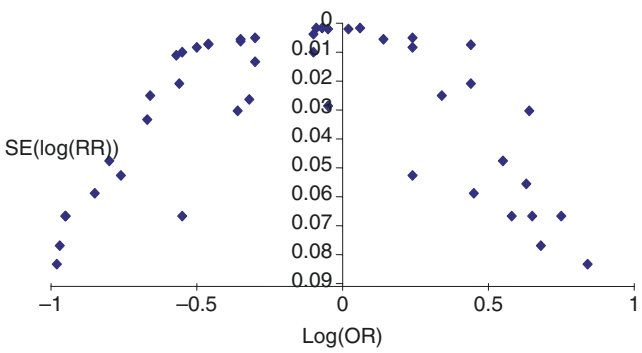

Fig. 80.1 Symmetrical funnel plot

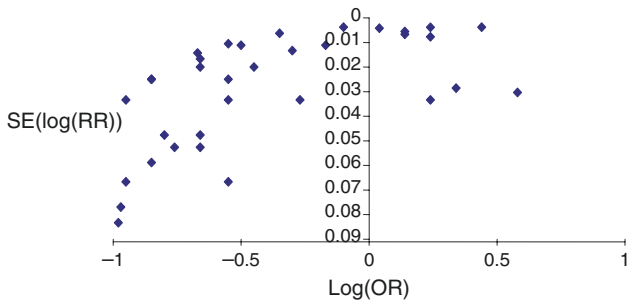

Fig. 80.2 Asymmetrical funnel plot

In absence of bias, the shape of the plot depends on the choice of the axes. Standard error was shown to be the best choice for the vertical axis because the expected shape in the absence of bias corresponds to a symmetrical funnel, straight lines to indicate 95% confidence intervals can be included, and emphasis of the plot is on smaller studies where bias is more likely. The only disadvantage, compared to other choices for the vertical axis, is that the axis has to be inverted to place the largest trials at the top of the graph [30, 31].

Visual evaluation of funnel plots may be subjective, and more formal statistical methods, to examine associations between the study effects and size, were proposed [32].

Duval and Tweedie have proposed the so-called "trim and fill" method. It is based on a rank data augmentation technique that adds studies to a funnel plot so that it becomes symmetrical [33]. Smaller studies at the bottom are omitted until the funnel plot is symmetrical (trimming). The trimmed funnel plot is used to calculate a summary estimate by standard meta-analysis approach. The trimmed studies are then replaced and their missing counterparts around the centre imputed or "filled". This provides an estimate of the number of missing studies and an adjusted summary estimate that is obtained including the "filled" studies.

Begg and Mazumdar proposed a rank correlation method that uses Kendall's tau to evaluate the association between the effect estimates and their variances [32].

Egger introduced a linear regression approach, which is equivalent to a weighted regression of the estimate on its standard error.

Egger's method is more sensitive than Begg's rank correlation approach, but the sensitivity of both methods is generally low in meta-analyses based on less than 20 trials [31]. Both Egger's regression method and the "trim and fill" method may be related to a great false-positive rate in detecting significant asymmetry of funnel plots. Furthermore Egger's method is known to be intrinsically biased [34].

Copas proposed a model in which the probability that a study is included in a meta-analysis depends on its standard error [35]. The model describes the process of study selection (publication bias) and evaluates the pooled OR for different parameter choices, which can be interpreted as the probabilities that a paper with a certain value of "standard error" is published (publication probability). For any given value of publication probability, Copas and Shi proposed a method to estimate the number of studies that were undertaken but not published and the correspondent reduction in the estimated risk. As there are not enough data to choose a single "best" model, the authors proposed a sensitivity analyses in which the value of the estimated risk factor is computed under a range of assumptions on the severity of the selection bias. The danger of the testing approach for funnel plot is the temptation to assume that, if the test is not significant, there is no problem, and hence the possibility of publication bias can be ignored. Copas and Shi argued that publication bias is endemic to all empirical research even if it is not evident from the funnel plot. Approaches, which try to estimate exactly how many studies are missing, are very hazardous, and graphical tests make assumptions that cannot be tested. The sensitivity analysis proposed by Copas and Shi monitors how sensitively the results depend on the assumptions using a model to describe the process of study selection, estimate the effect of interest for different parameter choices within this model and then check the fit of each estimate with the evidence in the funnel plot. The application of this method is quite straightforward because the authors published the S-plus routines, which is useful to carry out the calculations [35].

These statistical methods, which investigate the asymmetry of the funnel plot, try to estimate how big the impact of publication bias might be on the results. However none of them can be considered the ideal statistical method for assessing publication bias, and any method should be considered indirect and exploratory. Modelling assumptions used may heavily influence the estimates adjusted for publication bias. Many factors may be involved in the publication process, and it is difficult, if not impossible, to adequately model them. These methods may detect "missing" studies even in the absence of bias, adding and adjusting for nonexistent studies in response to funnel plot asymmetry arising from nothing more than random variation [30]. As a matter of fact their sensitivity is generally low in meta-analyses based on less than 20 trials [30, 36]. The tests are most useful for large meta-analyses and when there is a wide range of study size. However, based on the empirical type I error rates, a regression of logOR on sample size, weighted by the inverse of the pooled variance to allow for possible heterogeneity, is the preferred approach.

It was estimated that missing studies change the conclusions in less than 10% of meta-analyses, suggesting that publication bias, although widespread, may not be a major problem [37]. It is therefore wise to restrict the use of statistical methods that model selection mechanisms to the identification of bias rather than correcting it [38].

80.8 Some Examples of Meta-analyses and Pooled Analyses on Breast Cancer Risk and Survival

One of the first meta-analyses on breast cancer was published in 1988 by Longnecker et al. [39], in which the authors investigated the association between alcohol and breast cancer. They found a significant dose-response relationship both in case-control and in cohort studies, strongly supporting the detrimental effect of alcohol consumption on breast cancer risk. In contrast, in a recent meta-analysis investigating the association between serum 25-hydroxyvitamin D levels and cancer risk, we could not establish an association with breast cancer because of different results in case-control and cohort studies. In fact, we found a significant association only in case-control studies where a reverse causation could have biased the results [40].

One of the most cited pooled analysis [41] of individual patients data summarized results of 194 unconfounded randomized clinical trials of adjuvant chemotherapy or hormonal therapy for early breast cancer, assessing recurrence and 15-year survival. Owing to its large size, this study had enough statistical power to draw reliable conclusions about the long-term benefits of various breast cancer treatments.

Another widely cited pooled analysis [42] of data from 22 studies allowed a reliable estimation of the cumulative risk of breast and ovarian cancer in BRCA1 or BRCA2 mutations carriers, providing important information for genetic counselling. Information that none of the single study could have given with accuracy. As a final example, investigation of heterogeneity in another meta-analysis in BRCA1/2 mutation carriers allowed us to assess differences in the effect of oral contraceptive formulations by time. We found that formulations used before 1975 were associated with a significant increased risk of breast cancer but not more recent formulations [43].

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Trial Designs and Biostatistics for Molecular-Targeted Agents

81

William T. Barry

81.1 Introduction

Prospective clinical trials are a key element in the developmental process of investigational drugs for treatment of cancer. For the past 50 years, clinical trials have largely been segmented into investigations that are defined by the cancer's site of origin. For example, treatment paradigms for breast cancer patients have developed independently from those for patients with lung cancer. Early work in cancer biology has demonstrated that the disease is heterogeneous within a given cancer type, with some common features that allow for tumors to be classified by molecular phenotypes [1]. Our increased knowledge of the molecular alterations which occur and drive disease progression has also transformed drug discovery in oncology. Today, many new compounds have been identified which target specific defects or cellular pathways that are dysregulated in tumors. The discovery of many multiple classes of drugs has opened the door to the use of personalized medicine in treatment of the disease, whereby lines of therapy can be tailored to the molecular phenotype of the individual's tumor. Furthermore, the targeted genetic mutations and molecular phenotypes often occur across multiple cancer types [2]. This has led to the application of new clinical trial designs, where patient eligibility is not restricted by the anatomical origin of the tumors and molecular assays are incorporated to establish the biomarker status of all patients and to evaluate the effectiveness of drugs under the paradigm of personalized medicine.

Formally, the term biomarker has been broadly defined by the National Institutes of Health Biomarkers Definitions Working Group as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [3]. The biomarkers used in clinical cancer research today were originally discovered and developed within many different disciplines of academic medicine, whether pathology, radiography, immunology, or cancer genetics [4]. When utilizing a biomarker clinically, there is the added layer of complexity that there may be more than one laboratory assay available, and standardization can improve both the quality of testing and also the consistency of application in clinical research. At the same time, the assessment of biomarkers can be improved over time with development of new biotechnologies and further analytic validation. For instance, in 2013, the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) updated their recommendations for human epidermal growth factor receptor 2 (HER2) testing in order to incorporate new diagnostic strategies when measured by immunohistochemistry (IHC) and/or in situ hybridization (ISH) [5].

The clinical utility of any biomarker will depend on the type of information it provides about the disease state. Biomarkers in breast cancer can be categorized as measures of the risk of development of breast cancer, prognosis of individuals diagnosed with the disease, prediction of benefit to specific therapies, or measures of response to interventions. Many of the most well-characterized molecular phenotypes in breast cancer, however, provide multiple pieces of information about the disease process. This includes the genotype of BRCA1 and BRCA2 (risk, prognosis, and possibly prediction), HER2 gene amplification (prognosis and prediction), and expression of the Ki67 gene in tumor tissue (prognosis and response to therapy). Thus, when designing a clinical trial that incorporates a molecular biomarker, one must consider context and what specific clinical utilization is under investigation.

Biomarker research was traditionally conducted as correlative science to clinical trials where the primarily objective was to investigate safety and efficacy of a new drug or

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intervention. Guidelines have been issued on the collection and use of biospecimen for biomarker development and on assay requirements before their incorporation into prospective trials [6, 7]. A set of criteria, REporting recommendations for tumor MARKer prognostic studies (REMARK), was compiled in 2005 by an international committee to improve the quality and reproducibility of study results [8]. One advantage to retrospective investigations of biomarkers is that they allow for a variety of approaches common to observational studies, such as nested case-control designs that can be more efficient when trial populations are large and the clinical outcome or molecular phenotype is rare [9]. However, only prospective application of the biotechnology will fully evaluate the clinical utility of an assay [6] that depends on additional factors including accessibility of a biospecimen, quality control of specimen collection and laboratory procedures, and the feasibility of running assays in real time to make clinical decisions in a timely manner. In recognition that there are operational challenges and costs with prospective clinical trials, prospective-retrospective studies have been proposed where prospective processing of banked specimen could be run in an in silico manner to obtain high-quality evidence of clinical utility. In a report from the Institute of Medicine, *Evolution of Translational Omics: Lessons Learned and the Path Forward* [10], the validation of the Oncotype DX® assay in the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-14 is used as a case study of a prospective-retrospective evaluation from banked specimen. The investigators found that the assay was prognostic for disease recurrence and overall survival in patients with node-negative and estrogen receptor-positive tumors [11]. Further retrospective studies indicated that the assay was also predictive of benefit from adding chemotherapy to tamoxifen treatment in patients with higher risk of recurrence [12] and that the prognostic and predictive value extended to a study population of node-positive breast cancer patients [13]. Ultimately, a large prospective trial was required to prospectively validate the assay and to further refine its clinical utility and optimal thresholds for selecting between endocrine and chemotherapy-based regimen.

In this chapter, a series of trial designs are presented in detail for prospective biomarker-driven studies where the primary objective incorporates both the marker status of the study population and the efficacy of anticancer treatments. A description of some of the analytic approaches for biomarker-driven studies is provided, including statistical methods for fixed group sequential and adaptive designs. The complexity of breast cancer studies highlighted here ranges from investigations of a single agent with a paired biomarker to multiplex assays to assess a panel of molecular alterations and treatment strategies that incorporate multiple targeted therapies.

81.2 Trial Designs for Heterogeneous Populations

In 2009, the Biomarkers Task Force of the National Cancer Institute (NCI) Investigational Drug Steering Committee provided a set of definitions for biomarkers that are incorporated in a prospective manner into cancer clinical trials [14]. Standardized criteria were established that determine whether a marker has an *integrated* or *integral* role in the study design and conduct. Specifically, an integrated marker is performed in a trial "to identify or validate assays that are planned for [future] use," where "statistical design and analysis should be pre-specified" and "include complete plans for specimen collection, laboratory measurements, and analysis." Studies with integral markers have many of the same elements, but are also designed such that the assay must be completed before patients can proceed on the trial. Examples of integral biomarkers include those used to establish eligibility, determine patient stratification and randomization, or inform treatment assignment.

81.2.1 Biomarker-Based Trial Designs

Treatment trials which incorporate integral biomarkers generally fall into one of three classes: marker-stratified designs, marker enrichment designs, or a broader class of marker-directed designs [15]. Some of the unique elements and limitations of each design are discussed below.

81.2.1.1 Marker-Stratified Designs

Marker-stratified designs are studies where the biomarker-defined subgroups are incorporated into the trial as a stratification factor used during randomization. Equivalent allocation schemes are applied within each stratum so that the expected treatment assignment does not vary by biomarker status. A representative schema is shown in Fig. 81.1 for a study population that can be segregated into three biomarker-defined subgroups; in each stratum patients are randomized to receive one of two treatments. The distinction from integrated biomarker studies is that marker status is required at study entry, which allows for other design characteristics to be considered (e.g., capping the total sample size to each marker-defined subgroup). The scientific objectives and analysis plans of a marker-stratified design can take a number of different forms, as explored later in this chapter, but must be pre-specified and justify the sample size in accordance with the hypothesized relationship between biomarker status and efficacy of the treatment.

An example of a breast cancer trial that used the marker-stratified design is a randomized placebo-controlled phase III study to investigate the addition of lapatinib to letrozole as a

targeted strategy for patients with hormone receptor-positive metastatic breast cancer [16]. The study enrolled a total of 1280 patients in order to have a subset of 218 patients with HER2-positive tumors. The sample size of the subgroup was selected to be powered to detect a 55% improvement in progression-free intervals with the combination and to secondarily test efficacy in the total study population. The study demonstrated significant improvement with the combination in the HER2-positive subset which was attenuated in the overall population but remained statistically significant. However, based on a post hoc evaluation in the HER2-negative subset, the investigators rightly concluded "lapatinib plus letrozole failed to delay endocrine resistance in more than 750 patients with endocrine-sensitive, EGFR/HER2-negative disease." This trial result illustrates that marker-stratified designs and testing strategies require careful consideration of power and clinically meaningful effect sizes within and across subgroups.

81.2.1.2 Marker Enrichment Designs

Marker enrichment designs are studies where biomarker-defined subgroups are selected for participation in the trial. Selection could be based on a predictive marker of sensitivity to protocol treatment or based on a prognostic marker to identify higher- or lower-risk patients for whom clinical benefit with the treatment is hypothesized. With predictive biomarkers, enrichment designs are most appropriate when there is sufficient prior information to indicate that efficacy will only be seen in the molecularly defined subpopulation. In Fig. 81.1, the right-most schema applies an enrichment strategy by excluding patients that determined to be in [marker subgroup] 1.

A classic example of enrichment studies leading to approval of drugs has been the investigation of trastuzumab and other anti-HER2 therapies in breast cancer. The efficacy of adding trastuzumab to standard adjuvant chemotherapy was demonstrated in two randomized phase III trials: NSABP trial B-31[1] and North Central Cancer Treatment Group trial N9831.[2] A total of 3351 patients with HER2-positive disease were treated across the two studies and showed vastly improved disease-free survival with the addition of the targeted therapy [17]. Eligibility to both studies was established by local assessment of HER2 using standard criteria. But, in a follow-on report from B-31, central confirmation of HER2 status was performed, and discordance with local assessment was seen in about 10% of cases. Furthermore, a consistent level of benefit was seen with the addition of trastuzumab in every subset defined by centralized IHC or FISH [18]. This left open a scientific question of whether benefit of anti-HER2 therapy would extend to some patients that are not HER2-positive under ASCO-CAP guidelines. To investigate this further, a randomized phase III clinical trial NSABP B-47[3] has enrolled 3260 patients with intermediate HER2 gene amplification (IHC 1+ and 2+ that are negative by FISH) to evaluate whether trastuzumab will improve invasive disease-free survival. Enrichment designs have also been adopted in three trials of PARP inhibitors as treatment of metastatic breast cancer in patients harboring BRCA mutation [19].

81.2.1.3 Marker-Directed Designs

Marker-directed designs are studies where treatment assignment is determined by the integral biomarker. This type of design encompasses a broader class of studies, including trials where either a fixed or random assignment of patients is specific to one or more of the biomarker-defined subgroups and also where unequal randomization schemes are applied within each subgroup. For instance, the rightmost schema in Fig. 81.1 directs patients in [marker subgroup] 2 to receive treatment A, while those in [marker subgroup] 3 are randomized in an imbalanced fashion to the two treatment regimen.

An example of a marker-directed design being used in breast cancer research is the Trial Assigning IndividuaLized Options for Treatment (Rx), TAILORx,[4] to prospectively validate the prognostic and predictive value of the Oncotype DX® recurrence score (RS). Specifically, patients with the lowest risk scores (RS < 11) were directed to endocrine therapy alone, and those with the highest risk scores (RS > 25) were directed to chemotherapy. Patients in the intermediate range ($11 \leq RS \leq 25$), where equipoise still existed, were randomized to receive one of the two treatment regimens. Statistical analysis plans are to test for non-inferiority of endocrine therapy and secondarily to explore whether the upper ranges of RS are modifiers of any potential benefit from chemotherapy. A total of sample size of approximately 11,000 patients was selected to have an adequate number at intermediate risk to participate in the randomized component of the study.

81.2.2 Adaptive Designs

When considering the strengths and limitations of the biomarker-based studies described above, it is important to note that only the marker-stratified design provides direct evidence of the performance of the biomarker as a predictor of treatment benefit. However, a limitation of the design is that the sample sizes can be prohibitive when the prevalence of marker positive is rare. Conversely, a marker enrichment design cannot be used to validate the performance of the

[1] NCT00004067.
[2] NCT00005970.
[3] NCT01275677.
[4] NCT00310180.

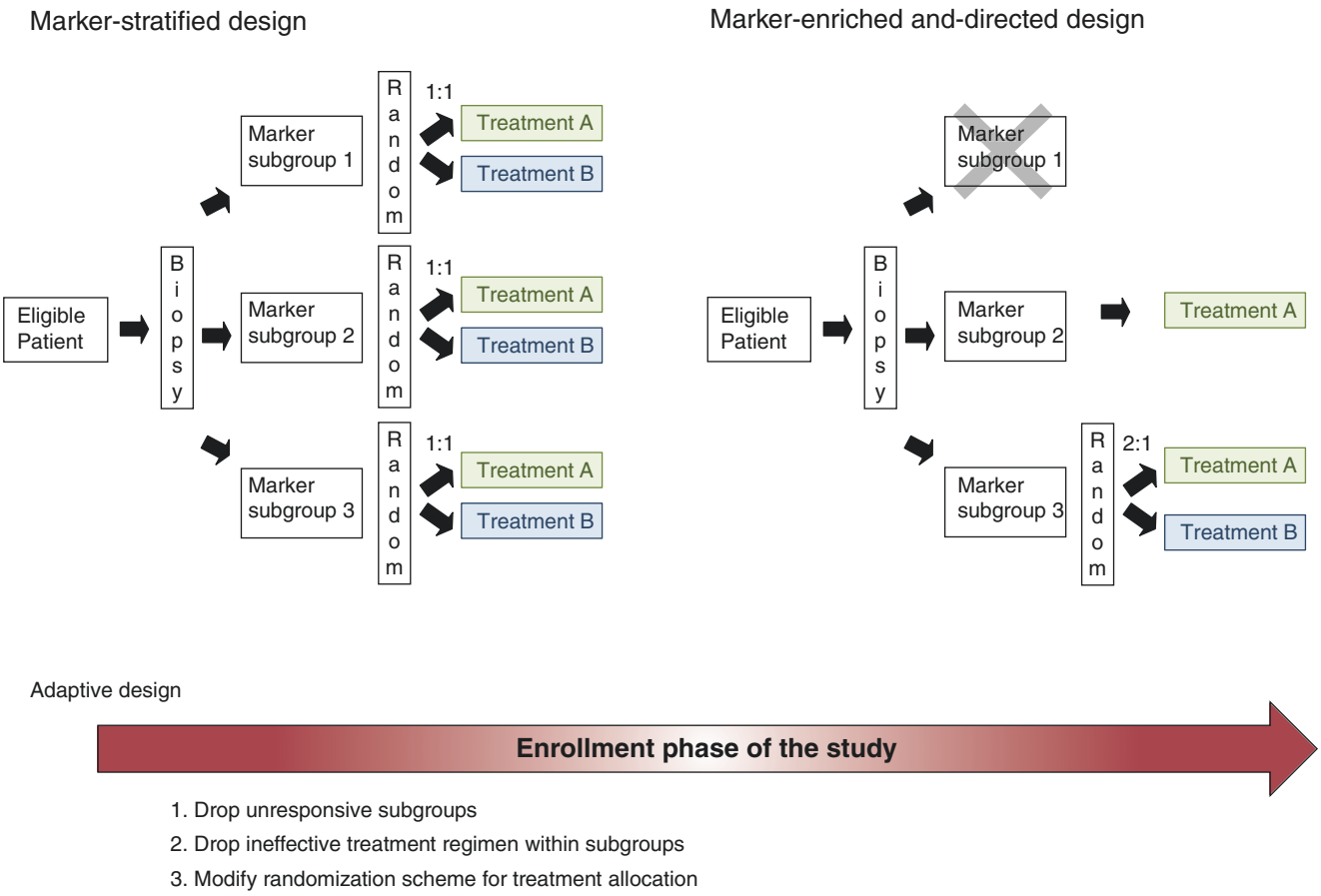

Fig. 81.1 Schema for biomarker-driven trials and elements of adaptive designs

marker, but is more efficient when treatment effects are strongly modified by the molecular phenotype [20]. Likewise, a marker-directed design can allow one to investigate different hypotheses within the marker-defined subgroups under one protocol. This distinction in operating characteristics will only hold for studies that are conducted as a single-stage analysis plan, and the theory and methodology of group sequential and adaptive designs can be applied in ways to allow a trial to transition over the course of investigation from a stratified design to an enrichment or directed design.

The designation that a trial is *adaptive* is applied whenever there is an iterative analytical plan that uses the accumulated information to make modifications to design elements. Some of the most common prospective studies with adaptive designs are phase I dose-finding studies, phase II and III studies with sample size re-estimation or response-adaptive randomization, and seamless phase II/III studies. To adapt a biomarker-driven trial, a sequential testing strategy can be used to modify a trial to (a) exclude a marker-defined subgroup where inadequate treatment benefit has been observed, (b) drop a treatment regimen in a subgroup-dependent manner, or (c) alter the ratio of randomization in a multi-arm trial. The schemas in Fig. 81.1 were selected in a way that one modification of each type would transition from the marker-stratified design to the marker-enriched and marker-directed design shown. The iterative process could be performed as a group sequential test at fixed points in the enrollment period or under a continual assessment plan.

The analytic plans to adapt biomarker-driven trials can be based on standard criteria used to evaluate the efficacy of drugs, such as the decision rules for stopping a trial early for futility. For a single-arm phase II trial without a concurrent control, the predominant approach is the Simon two-stage design that controls error rates while minimizing sample size requirements [21]. Likewise, early stopping for futility is often a required element of phase III randomized controlled trials [22]. A natural extension to biomarker-driven studies is to define staged tests of clinical activity within subgroups [23], and the study population would be enriched in the latter part of the trial if clinical activity is limited to one marker-defined subgroup. This simple extension can be burdensome when having to conduct interim tests at fixed points of enrollment within each subgroup. Thus, some designs perform unselected enrollment in stage one and evaluate efficacy in the randomly sized subpopulations [24]. Stopping rules can also be based on other parameters related to biomarker status and treatment effects, such as the predictive value of the biomarker [25]. Adaptive methods have also been developed for

a continuous biomarker without established threshold(s), where accumulated information on the predictive value is applied to a series of group sequential tests to transitioning from an all-comers design to enrolling only a marker enrichment population for final testing of efficacy [26]. The same rules can be extended to non-comparative multi-arm trials so that future patients are directed toward drugs with the greatest benefit observed.

Lastly, the use of response-adaptive randomization can also allow for a gradual and seamless transition from marker-stratified to marker-directed assignment over the course of the trial. However, such adaptive designs require careful development and monitoring to achieve desired operating characteristics [27]. This adaptive methodology has been applied in the trial Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer (I-SPY 2),[5] with the motivation that this seamless process can ultimately result in more accurate and faster drug development. To further achieve this goal, once a drug graduates from the I-SPY 2 trial to a phase III confirmatory study or drops from the study for futility, it is replaced by new investigational agent. For both response-adaptive randomization and group sequential testing, early end points of efficacy are needed to maximize efficiency in terms of number of patients enrolled and total study length. For this reason, the neoadjuvant paradigm and measurement of treatment effects using pathologic complete response has also accelerated drug development in breast cancer.

81.2.3 Basket and Umbrella Trials

With our current knowledge of the genomic diversity in cancer, and with the discovery of a variety of drugs that target specific molecular phenotypes, there has been a push to change the paradigm of how clinical cancer research is conducted. Whereas traditional drug development involved testing a single drug with or without a companion diagnostic, a number of clinical trials have recently been developed that enroll a diverse population of patients to evaluate multiple targeted treatments (Table 81.1). The motivations are both scientific, in seeking to answer broader questions of efficacy under precision medicine, and pragmatic, in gaining operational efficiencies from incorporating multiple investigations under a single protocol. The terms "basket" and "umbrella" have been used to evoke the manner in which the trial flows patients into the series of investigations, and each design requires integral biomarkers to establish the patient status at study entry.

Table 81.1 Biomarker-driven trials with multiple molecular targets or investigational drugs

Trial	Design	Study population	Target gene alterations or protein expression[a]	Investigational drugs[a]
NCT01953926	Basket (non-randomized)	Solid tumors	EGFR, HER2, HER3	Neratinib
NCI-MATCH NCT02465060	Umbrella (non-randomized)	Solid tumors or lymphomas	ALK, ROS1, BRAF, EGFR, HER2, NF2, cKIT	Afatinib, AZD9291, crizotinib, dabrafenib, sunitinib, T-DM1, trametinib, VS6063
Lung-MAP[b] NCT02154490	Umbrella (randomized)	Squamous cell lung cancer	PI3K, CDK4, CCND1, CCND2, CCND3, FGFR	AZD4547, ipilimumab, nivolumab, palbociclib, taselisib
I-SPY 2 NCT01042379	Umbrella (randomized adaptively)	Breast cancer	ER, PR, HER2, MammaPrint®	ABT-888, AMG 386, ganetespib, ganitumab, metformin, MK-2206, PLX3397, neratinib, pembrolizumab, pertuzumab, T-DM1, trastuzumab
SAFIR 02[b] NCT02299999	Marker strategy (randomized)	Breast cancer	AKT, AR, EGFR, HER2, MEK, mTOR, PARP, VEGF	AZD2014, AZD4547, AZD5363, AZD8931, bicalutamide, olaparib, selumetinib, vandetanib
SHIVA [30] NCT01771458	Marker strategy (randomized)	Solid tumors	KIT, ABL1/2, RET, PI3KCA, AKT, mTOR, RICTOR, RAPTOR, PTEN, STK11, INPP4B, BRAF, PDGFRA/B, FLT3, EGFR, HER2, SRC, EPHA2, LCK, YES1, ER, PR, AR	Abiraterone, dasatinib, erlotinib, everolimus, imatinib, lapatinib, letrozole, sorafenib, tamoxifen, trastuzumab, vemurafenib

[a]Target molecular phenotypes and investigational drugs for each trial were obtained from https://clinicaltrials.gov/ as of Jan 1, 2016
[b]Trial design and investigational drugs was obtained from updates posted at http://www.lung-map.org

[5]NCT01042379.

The label of a "basket trial" is typically applied when a single drug is under investigation, and patients from a variety of disease settings are enrolled into the study. Clinical activity is then evaluated separately in each basket defined by traditional disease characteristics. For newer classes of drugs, including immunotherapies, the basket design has become popular for early phase investigations before conducting multiple definitive randomized studies in individual disease types. For targeted drugs, a basket trial allows one to enroll a diverse study population who share common molecular phenotype thought to impart sensitivity to the agent. As one example, a non-randomized phase II study is evaluating neratinib monotherapy in solid tumors known to carry mutations or amplifications in one of the human epidermal growth factor receptor genes: EGFR, HER2, and HER3.[6] The individual baskets are defined by combinations of disease type and molecular phenotype (e.g., gastric cancers harboring a HER2 mutation, or any solid tumor with a HER3 mutation).

Conversely, an "umbrella trial" will enroll a study population defined by generic disease characteristics and then use a set of integral biomarkers to direct patients into substudies to evaluate a variety of experimental therapeutics. Two of the first umbrella trials were sponsored by the NCI: the ALCHEMIST trial[7] screens several thousand patients with operable, non-squamous, non-small cell lung cancer to select those with ALK rearrangement or EGFR mutations for randomization to targeted therapy versus standard of care. The Lung-MAP trial[8] used the genomic profile of advanced squamous tumors to match patients to randomized sub-studies of targeted therapy or to evaluate a new combination of immunotherapies in unmatched individuals. The use of umbrella designs was extended more recently in the NCI-MATCH ("Molecular Analysis for Therapy Choice") trial,[9] to perform genomic profiling of any consenting adult with a solid tumor or lymphoma and to allocate patients with a matching alteration to a drug within a portfolio of targeted investigational products. The primary evaluation of a drugs' efficacy will be in all treated patients, and not in separate baskets defined by the type of cancer. Lastly, the I-SPY 2 trial can be characterized as an umbrella trial, in addition to using outcome-adaptive randomization, because the comparisons of investigational drugs to standard of care are analyzed and reported separately. The first treatment regimens to report positive findings from the trial have been the combination of veliparib and carboplatin for triple-negative disease, neratinib for HR−/HER2+ disease, and the AKT inhibitor MK-2206 in HR− and/or HER2+ disease.

[6]NCT01953926.

[7]NCT02194738.

[8]NCT02154490.

[9]NCT02465060.

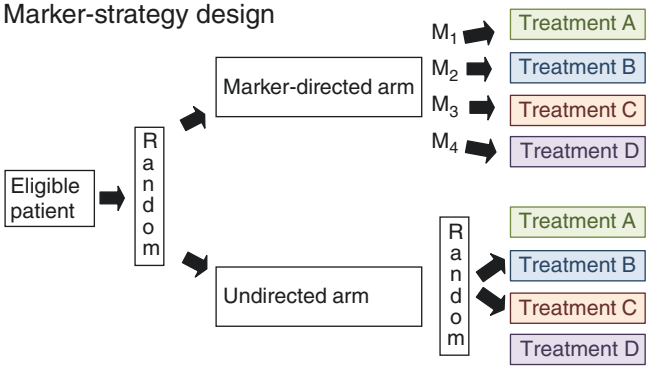

Fig. 81.2 Schema for a marker strategy design

81.2.4 Biomarker Strategy Designs

While the traditional drug development process has been to investigate a single treatment regimen with or without using a molecular biomarker to define the target population, different approaches have been proposed for evaluating the clinical effectiveness in the era of precision medicine including the biomarker strategy design (Fig. 81.2). Here, the experimental arm utilizes an algorithm for directing patients to receive one of a portfolio of drugs according to their biomarker status. A randomized controlled study could use several different strategies to assign treatment in the other arm: a different set of drugs that are standard of care, random allocation to the drugs in the portfolio, or physician's choice naïve to the patient's biomarker status. Outcome can then be compared between the two randomized populations without direct consideration of the individual treatments received in each arm. The trial could be conducted in a narrow patient population using a small number of directed therapies or in a broader setting when a suitable treatment algorithm can be constructed for a more heterogeneous population.

The marker strategy design has been implemented in the SAFIR 02 trial[10] for patients with metastatic breast cancer. Genomic profiling was first piloted in a non-randomized screening study, where investigators found that 195 of 423 patients (46%) had a target genomic alteration detected [28]. In SAFIR 02, patients who begin treatment with standard chemotherapy are screened for 51 molecular alterations which are matched to a targeted therapy. Patients with a detected alteration are then randomized after a fixed number of cycles to continue on standard of care or to switch to the target monotherapy. The primary objective is to compare the progression-free survival of patients on each arm. An example of a marker strategy design used in a broader setting is the SHIVA trial[11] which enrolled patients with any solid tumor deemed refractory to

[10]NCT02299999.

[11]NCT0177145.

standard of care for the disease type [29, 30]. Patients were screened for a number of genomic and proteomic alterations that are targeted by ten treatment regimens. When an alteration was detected, patients were randomized either to receive an agent under the protocol-specified treatment algorithm or to receive treatment by physician's choice. The study was designed to include a total of 200 patients with alterations and powered to detect a 40% improvement in progression-free survival under an assumption of a homogenous treatment effect. After screening 741 patients, targeted alterations were detected in 293 individuals and 195 were randomized to treatment (breast cancer was the most common disease type, n = 40). The primary analysis failed to identify a significant improvement in progression-free survival. For both marker strategy designs, the treatment regimens vary between the experimental and control arm which leads to confounding between randomized assignment and the effects of the individual treatments. For this reason, it is difficult to discern whether the negative results from the SHIVA trial are attributed to the marker-directed strategy, efficacy of the ten targeting drugs, or other aspects of the design and enrolled study population.

81.3 Statistical Methods for Biomarker-Driven Trial Designs

One of the challenges in developing a biomarker-driven trial is to distinguish the overall hypothesis and scientific goals of the study. This will not only drive the design but also frame the analysis plan for primary and secondary objectives. All study plans should be described in the trial protocol that governs study conduct. The section on statistical considerations should be developed according to the study objectives and provide a justification of the sample size, the statistical methods, and detail on the set of inferences planned to avoid any research biases from testing additional post hoc hypotheses.

In a treatment trial that assumes a homogenous patient population, the primary hypothesis is simply a question of whether outcomes are improved when given a new drug relative to a randomized or historical control. For biomarker-driven trial designs, the hypothesis might be reframed to (a) ask questions of improved outcomes conditioned or modified by biomarker status, (b) validate the predictive or prognostic value of the biomarker, or (c) both. To investigate the causal relationship between a drug intervention and outcomes, randomized controlled trials provide the highest level of evidence of the treatment effect and its modification by baseline factors. The same holds for biomarker-driven studies, and estimates will be unbiased assuming that complete and error-free molecular information is obtained before treatment begins. Finally, in order for randomization to provide the necessary properties for causal inference, analyses should follow the *intention-to-treat* principle and analyze all subjects according to their assigned intervention, and integral biomarker studies will prevent any systematic errors from specimen processing from introducing bias.

81.3.1 Evaluations of Biomarkers and Drugs

Before the utility of a biomarker is ready to be assessed in a prospective clinical trial, the assay must first be demonstrated to have analytic and clinical validity. Analytic validation of a laboratory test establishes that its performance characteristics meet the requirements for clinical application, including accuracy and precision. Accuracy can be quantified in terms of sensitivity and specificity from a collection of specimen where a dichotomous test result is compared to a "gold-standard" measurement of the underlying phenotype. For laboratory tests that return a continuous score, performance as a binary classifier can be displayed using a receiver operating characteristic (ROC), and indexes such as Youden's J statistic, $J = \text{sensitivity} + \text{specificity} - 1$, can be used to select an optimal threshold.

The precision of a laboratory assay be can be evaluated under two scenarios: *repeatability*, when all efforts are made to keep conditions constant (e.g., instruments, operator, and time point of processing), and *reproducibility* across conditions (e.g., laboratories, platforms, or experiments). In developing the MammaPrint® assay based on the original 70-gene signature discovered with Agilent microarrays, a paired experiment was performed using a collection of banked specimens demonstrating a Pearson correlation >0.99 between the two platforms [31]. For cancer biomarkers, additional sources of variation need to be considered that may affect precision, such as spatial heterogeneity of the tumor, environmental factors (e.g., fasting), or time-varying effects of the disease and/or natural process as hypothesized for Ki67 expression levels across menstrual cycles [32]. For experiments with a variable number of unordered replicates, the intraclass correlation coefficient (ICC) is a useful statistic for quantifying the agreement in continuous marker levels, and Donner and Eliazew provide a sample size calculation for designing studies to assess reliability which depends on the number of replicates per sample and desired type I and II error when testing against a null ICC [33].

The clinical validity of a biomarker extends from the accuracy and precision of the assay versus a gold standard to the association with health outcomes that are related to the drug efficacy and treatment decisions. For outcomes that can be represented as a binary variable (e.g., radiographic response), the probability of an event can be parameterized as π_j where $j = 1, 2 \cdots m$ represents each biomarker subgroup. The null hypothesis of no association between outcome and biomarker status is $H_0 : \pi_1 = \pi_2 = \ldots = \pi_m$. The observed data from a study can be summarized in a $2 \times m$ contingency

table, and inferences can be made using Fisher's exact test. In cancer, clinical outcomes are frequently measured as the time interval until a disease event occurs, whether a disease-free interval until recurrence, time to radiographic evidence of progressive disease, or overall survival until death from any cause. In a clinical trial, the study timeline and data collection and the underlying disease process will not allow for all patients to be followed until an event occurs. For patients where an event is not observed, the end point is right censored at the time point at which the subject was last known to be event-free. Methods in survival analysis allow one to estimate the distribution of time-to-event data as a survivor function, $S(t)$, and to compare the outcomes in either a randomized trial or marker-defined subgroups against a null hypothesis, $H_0: S_1(t) = S_2(t)$ for all $t > 0$. Analysis plans can utilize parametric methods to estimate $S(t)$ and conduct hypothesis testing, such as the exponential model with the assumption of a constant hazard, λ, over time such that $S(t) = e^{-\lambda t}$. However, nonparametric methods are more commonly used in the analysis of breast cancer trials, including the Kaplan-Meier product-limit estimator for $S(t)$ and the log-rank test for two-sample comparisons with the null hypothesis stated above.

When evaluating the prognostic value of a biomarker, the association to disease outcome is estimated using a regression model. REMARK criteria include the recommendation to use multivariable models to discern the independent prognostic effect of a biomarker after adjusting for other known prognostic disease and patient characteristics. For time-to-event end points, the semi-parametric Cox proportional hazard model can be used to specify the functional relationship between covariates in the model, but underlying hazard function, $\lambda_0(t)$, remains distribution-free. As part of the validation of the Oncotype DX assay, the prognostic value of the recurrence score remained significant after adjusting for clinical factors including tumor size and nodal status [34]. The first set of results from the TAILORx trial demonstrated that the low-risk cohort had a very low rate of recurrence 5 years out from study entry [35]; however, as a marker-directed design, these data do not allow for the prognostic value of the classifier to be validated until outcomes are reported for the patients in the intermediate-risk cohort that were randomized to endocrine therapy.

For a predictive biomarker to be evaluated as a companion diagnostic to a targeted treatment, the FDA recommends prospective validation using a test of interaction between treatment effects and biomarker status. This approach could be conducted with an integral biomarker as part of a marker-stratified design, as an integrated biomarker to a study that enrolls all-comers, or a retrospective-prospective study using archived specimen, but requires a randomized controlled study of treatment. Reporting guidelines should follow the CONSORT with additional issues relating to the marker assays incorporated from the REMARK criteria. The parameter of interest in an interaction test is a multiplicative term in the linear predictor a multivariable regression model. In the simplest case of a categorical marker with two levels and a two-arm randomized trial, the parameter is given as β_3 in the following linear predictor:

$$\log(\lambda(t)) = \log(\lambda_0(t)) + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2,$$

where covariates for the treatment assignment and biomarker status are x_1 and x_2, respectively. The test is constructed against the null hypothesis, $H_0: \beta_3 = 0$. One challenge with an analysis plan to test for an interaction is that a larger sample size is needed to establish statistical significance relative to a main effect of the same magnitude, by a factor of $(p_1\, p_2)^{-1}$ where p_i is the proportion of patients in the i-th marker subgroup. Thus the minimum fold increase is under an idealized situation that the prevalence of marker-positive patients is exactly half of the study population [20, 36]. For this reason, alternative testing strategies have been proposed which perform multiple comparisons of treatment effects conditional on biomarker status, but it should be remembered that the inferences drawn from these analysis plans do not validate the predictive value of a biomarker for specific treatment benefit.

81.3.2 Multiple Comparisons

One of the important considerations for a biomarker-driven study is the consequence of any multiple testing driven by the complexity of the research question. For a simple biomarker enrichment design, this is less relevant because the primary evaluation of the treatment effect is in the entire study population. Likewise, for biomarker-directed designs, the basis for accounting for multiple testing will depend on how primary and secondary objectives are specified. If hypothesis is framed as co-primary objectives about the efficacy of each marker-directed therapy, the common procedure to control the family-wise error rate (FWER) of at least one false-positive finding is to make a Bonferroni correction to the p-values of each test: $p_j \le \alpha/m$ for all $j = 1, 2 \cdots m$. For biomarker-stratified designs, alternative testing procedures to the Bonferroni correction have been proposed to control study-wise false discovery. Freidlin et al. review the different methods of sequential or parallel assessment in the overall population and biomarker subpopulations and comment on the strengths and limitations of each [37]. This includes the testing method used in the phase III of lapatinib to letrozole in hormone receptor-positive metastatic breast cancer mentioned above, where the testing method was to evaluate sequentially efficacy in the HER2+ subgroup and then in the overall study population [16].

Other experimental approaches have been developed for when a biomarker is not sufficiently established to be integral or to be prospectively validated. For instance, the "biomarker-adaptive threshold" design has been proposed for when an assay has been analytically validated, but there is not a cut point defined for clinical utilization [38] and an "adaptive signature" design when a classifier remains to be developed from a multiplex assay [39]. In spite of the naming conventions to each method, analysis plans are conducted retrospectively once clinical outcomes are known for the study population.

81.3.3 Bayesian Statistics for Adaptive Designs

For adaptive designs, the use of Bayesian statistics is a natural approach to model the desired flexibility in design as parameters and to compute their posterior probabilities based on the accumulated data. Bayesian inference plans for clinical trials, whether fixed or adaptive, have used hierarchical models to evaluate treatment effects across a heterogeneous population. Under the formulation of Kass and Steffey [40], disease outcomes in a study population can be modeled as a random vector, y_n, which is conditionally independent given the parameters, θ. By conditioning on hyperparameters, φ, at the next level of the hierarchy, the individual $\{\theta_i\}$ are identical and independently distributed. Then, the elements of y_n will have a common probability model, p:

$$p(y_n|\theta) = \prod_{i=1}^{n} p(y_i|\theta_i)$$

$$p(\theta|\varphi) = \prod_{i=1}^{n} p(\theta_i|\varphi)$$

This hierarchical model was used in single-arm cancer clinical trials in order to borrow information across subgroups when evaluating efficacy [41, 42]. Likewise, the model has been implemented in randomized phase II studies investigating multiple experimental drugs [43]. In I-SPY 2, an experimental drug, say treatment A, "graduates" in a given marker-defined subgroup, x_j, if there is at least an 85% predicted probability of success in a future phase III trial that randomizes 300 patients with phenotype x_j between A and a standard of care, treatment B. The Bayesian probability of future success depends on the posterior distribution $P(\pi_{A,j} - \pi_{B,j} \mid \text{data})$ derived from the information accumulated in I-SPY 2. Conversely, if there is less than a 10% predicted probability of success in every biomarker subgroup, a drug can be dropped from I-SPY 2 for futility. The trial also performs response-adaptive randomization, using as input the posterior probability a drug is superior to all others within the j-th subgroup $P(\pi_{A,j} > \pi_{B,j}\ ,\ \forall B \neq A \mid \text{data})$. A key design feature of the I-SPY 2 trial which can improve efficiency of global drug development in breast cancer is that new drugs can be brought into the study.

Conclusions

In summary, with the number of genomic alternations that have been identified in cancer being targeted by novel therapies, we are undergoing a dramatic paradigm shift in the drug development process. Biomarker-driven trials are necessary to identify the subset of a heterogeneous population that will be sensitive to each targeted drug and to develop strategies for treating individuals under the paradigm of personalized medicine. The design and statistical analysis plans of every biomarker-driven trial should be framed around a hypothesized relationship between treatment effects and molecular phenotypes and establish how study-wise error will be controlled with any multiple comparisons. Umbrella and basket trials allow for efficient approaches to testing new therapies and bridging across the anatomic types of cancers to build study populations with similar molecular phenotypes. Adaptive designs play a specific role in biomarker-driven trials for transitioning from stratification of a general population to enrichment when assays are shown to accurately predict treatment benefit. Integral biomarker studies have an increased operational burden of performing assays and collecting results in real time, but more closely mirror the utilization of biomarkers for making treatment decisions in a personalized manner.

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Bioinformatics for Clinical Use in Breast Cancer

82

Fabrizio Bianchi

82.1 Background

Bioinformatics embraces a multitude of fields including informatics, computer science, statistics, mathematics, and engineering, which are focused on the analysis and interpretation of biological data. In the recent years, bioinformatics steadily acquired a prominent position in cancer research due to the massive amount of data produced by genomic screening studies (i.e., microarray and next-generation sequencing based) of tumor samples [1]. For example, the Tumor Cancer Genome Atlas (TCGA), which is one of the biggest and most comprehensive effort to the understanding of the cancer molecular profile, performed gene expression, mutation, copy number variation, methylation, and protein profile analysis, for a total of 11,077 cases spanning 34 different tumor types (December 2015 update) [2]. This is a massive amount of *-omics* data which required the development of large computing infrastructures, software, and ad hoc databases, in order to manage data and make it publicly available to research community.

With the advent of next-generation sequencing (NGS), the amount of data produced in a single sequencing experiment expanded up to a ~10,000-fold. For example, the sequencing of BRCA1/2 genes using standard Sanger method produces ~15 MB of data, while in an NGS analysis, the amount of data produced are in the range of 8 GB for a whole-exome sequencing analysis (WES) to ~150 GB (30× coverage; 3.6 billion reads; 90 billion nt; FASTA format) for a whole-genome sequencing (WGS) analysis. Therefore, for running an NGS profile study of ~200 samples, the space required is about 1.6 terabytes (TB) for WES and 30 TB for WGS, which is far beyond the actual capabilities of data storage and computational power in a personal computer. As an example, the "Hawkey" stand-alone software developed by Michal Schatz in 2005 [3], which allows researchers to investigate mutations in the genome to confirm a specific phenotype, literarily collapsed when the massive sequencing information coming from an NGS sequencer was loaded into PC memory before visualization [4]. This prompted computer programmers and bioinformaticians to rethink about the way to manage large *-omics* datasets and perform analyses by skipping as much as possible local computing resources. The Galaxy platform is an excellent Web-based platform developed by Penn State, Emory University, and other institutions that allows uploading of *-omics* data to a public server and use remote computational power to perform a multitude of bioinformatics analyses [5], including NGS data alignment, genetic variation analyses, and downstream analysis to dissect molecular mechanisms (https://galaxyproject.org). Another powerful Web-based platform is the Cancer Genomics Browser (https://genome-cancer.ucsc.edu; [6]) that hosts an intuitive Web interface to interrogate several large *-omics* datasets of cancer samples (including TCGA), to look for cancer-relevant molecular alterations. A list of tools available for all level -omics data analysis can be found in the "Omics Tools" website (http://omictools.com) that include technologies, applications, and analytical steps, for a total of 10,738 tools. Importantly, most of these are freely available and do not require particular bioinformatics skills.

A new emerging technology that allows intensive computational analyses without a sizeable computer infrastructure is cloud computing. Cloud computing relies on external powerful IT infrastructures that offer the user the possibility to hire, at competitive costs, the required computational power and data storage and also to configure shared computing resources for custom data analyses [7]. Consequently, cloud computing represents an important partner of *-omics* data analysis software. For these reasons several computer scientists are now adapting existing tools and developing

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new ones to perform bioinformatics on remote cloud computer clusters [8]. However, a critical aspect for cloud computing is the Internet speed connection that could become a bottleneck during data transfer, particularly when dealing with big dataset [7]. A way to skip such limitation is to physically send the hard disk drive (HDD) to third-party computer infrastructures, but this may be complicated when data contain sensitive information (such as in the case of clinical and genetic information). Privacy and data protection remains a relevant issue in cloud computing, despite most cloud-computing services providers offer encrypted data transferring protocols to safeguard data during upload from remote Internet server.

82.2 Bioinformatic Approaches to Interpret Breast Cancer Molecular Heterogeneity

Breast cancer has been one of the first tumor types to be analyzed using high-throughput screening technologies, such as microarray. In the early 2000s, landmark papers described the first high-density microarray gene expression profiles of cohorts of breast cancer patients and the discovery of diagnostic/prognostic gene expression signatures [9–11]. Concomitantly, numerous bioinformatic approaches for the analysis of microarray data were proposed either by adapting previous mathematical/statistical methods or by developing new ones. For example, hierarchical clustering analysis is one of the most frequently used method for interpreting molecular profile data, which was introduced in 1960s by Ward, J. H. Jr. in a pioneering study [12]. Joe H. Ward developed a method that allowed clustering of elements based on their maximal similarity considering their characteristics, thus forming groups in a hierarchical structure that may be useful to define novel types/subtypes [12]. The method was suggested for use in large-scale studies ($n > 100$) which fits well in a typical experiment of microarray expression profile screening. Indeed, after nearly 40 years using a very similar hierarchical clustering procedure, Perou et al. discovered that breast tumors could be subdivided in different subtypes based on the expression profile of a set of genes, i.e., the "intrinsic gene subset" [10]. The existence of breast cancer subtypes was then validated by other studies [11, 13] which confirmed at least four subtypes: the luminal A, luminal B, triple negative/basal, and HER2. Importantly, these subtypes display different metastatic behavior, from rather indolent tumors (luminal A) to very aggressive disease (basal/HER2) [11, 14]. Based on these discoveries, a prognostic model named "PAM50" was developed. PAM50 is a qRT-PCR /nCounter-based prognostic test [15, 16] which, by measuring the expression profile of a 50-gene signature, stratifies breast cancer patients in the four tumor molecular subtypes. PAM50 is used and commercialized as prognostic test to identify patients at high risk of recurrence (basal, luminal B, and HER2) who may be treated by adjuvant chemotherapy [17–19]. A similar prognostic test based on another gene expression signature is Oncotype DX [20]. Oncotype DX is a 21-gene qRT-PCR assay (16 cancer related and 5 used as reference) involved in cell proliferation, invasion, and signaling. Scientists developed an algorithm to calculate a "recurrence score" (RS) which combined gene expression data of the 16 cancer-related genes, weighted considering their expression correlation (calculated by hierarchical clustering and principal component analysis), to identify "who needs more than 5 years of tamoxifen among women diagnosed with axillary node-negative and estrogen receptor-positive breast cancer" [21]. This genomic test stratifies breast cancer patients in three main groups: patients at high risk of recurrence (treated by chemotherapy followed by hormonal therapy), low-risk patients (treated with hormonal therapy), and intermediate-risk patients. A recent study found that PAM50 and Oncotype DX test results are in partial agreement particularly in those patients classified as "intermediate-risk" patients [22]. Therefore, it would be mandatory a refinement of such genomic tests before their application in the clinical routine; indeed, no clinical decisions can be taken when a patient is classified as an "intermediate risk."

Beyond hierarchical clustering, several other gene-clustering methods were developed in the last years for gathering more insights in genomics data [23, 24]. A limit of these clustering algorithms is their trend to consider genes/proteins as independent entities, not taking into account that biological functions are the results of complex molecular interactions. Thus, a variety of bioinformatics tools and databases of biological information were shaped to join set of genes sharing similar functions and altered in a particular pathological condition. This is relevant when the research goal is not "merely" the identification of diagnostic/prognostic cancer biomarkers, but also the identification of molecular functions that are potentially druggable.

82.3 System Biology Approaches for the Inference of Gene Regulatory Networks

Recently, system biology approaches to *-omics* data were shown to be accurate enough to identify gene regulatory networks (GRN) that represent molecular mechanisms involved in human disease [25]. System biology is a research field focused on computational and mathematical modeling of complex biological systems. The current availability of large datasets of genomic information is bolstering the identification of complex interactions of genes/proteins using gene regulatory network inference (GRNi). In cancer research, the

application of GRNi using microarray expression datasets resulted in the discovery of mechanisms that explained the onset and progression of different tumors, including breast cancer [26]. This has led also to the identification of novel potential druggable targets [27]. Different mathematical and statistical methods were successfully applied to infer GRN from gene expression data [28–33]. All of these methods assume that gene/protein functional relationships are characterized by linear or nonlinear correlations in the experimental data. A linear correlation is observed when the ratio of change among variables is constant; conversely, a nonlinear correlation is observed when the ratio of change is not constant (Fig. 82.1a). Of course, the identification of nonlinear functional relationship is more difficult. Recently, bioinformatic tools for GRNi based on mutual information (MI) has been successfully applied in cancer research [30, 32]. MI captures nonlinear relationships between variables (genes) in addition to positive and negative linear correlations [32, 34, 35], by measuring the amount of information (i.e., entropy) gained about a random variable (gene X) when considering another random variable (gene Y). In the case of two independent genes, the expression profile of X does not give any information about the expression profile of Y, so MI is equal to zero. On the contrary, in the case of dependency of two genes, knowing the expression profile of X, it is possible to determine the expression profile of Y and vice versa (X is a deterministic function of Y; Y is a deterministic function of X); thus MI is equal to one. In another embodiment, the way a gene X is regulated in a particular condition has the same uncertainty (i.e., entropy) after gene Y is known. The first attempt to build GRN by using MI was pursued by Butte and Kohane [36]. Two genes were graphically linked using edges only if they have an MI score above a specified threshold. Statistical significance of MI scores is then calculated using permutation test [37]. A critical aspect in GRNi could be the case of indirect relationship among genes which is a frequent condition in molecular mechanisms (i.e., a signaling cascade). In the ARACNE algorithm, the problem of indirect interactions was elegantly tackled using the data processing inequality (DPI) measure [32]. The ARACNE algorithm starts assigning pairwise connections to genes according to the computed MI scores. Before assigning connections a threshold of significance of the computed MI score is established. Then, the algorithm compares all the possible triplets (gene X, gene Y, and gene Z) and removes the edge with the smallest MI value representing an indirect interaction (Fig. 82.1b). Using ARACNE, highly interconnected genes in GRNs, also called as "gene hubs," were proposed to function as master regulators of cancer-relevant signaling pathways (Fig. 82.1b) [38, 39]. Of note, NGS screening studies are adding additional information which could help to identify cancer driver genes within these GRNs and design novel molecularly targeted therapies.

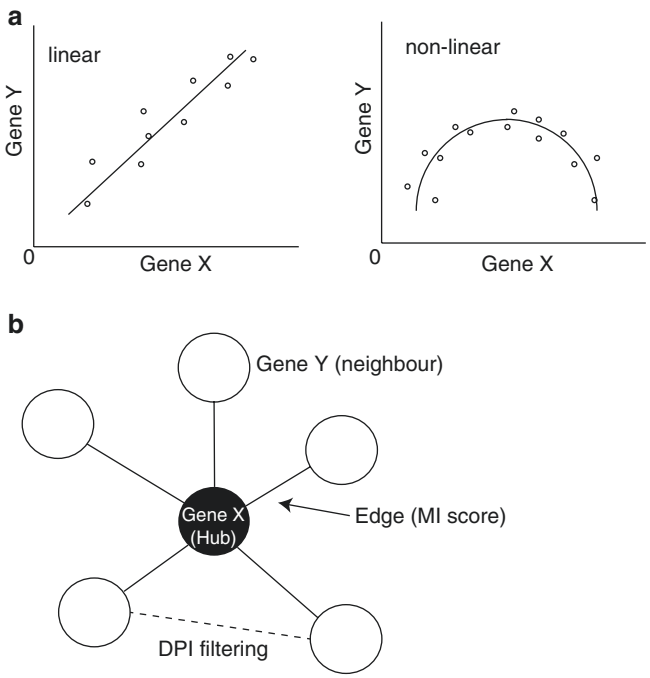

Fig. 82.1 Inference of gene regulatory networks (GRNi). (**a**) Examples of linear and nonlinear correlation between two genes. (**b**) A gene regulatory network

Conclusions

Besides the so-called inter-tumoral variability which characterizes the four breast cancer molecular subtypes, it is now evident that a high heterogeneity is present within the same tumor, i.e., the "intra-tumoral" variability. Navin et al. elegantly demonstrated by single-nucleus sequencing that in primary breast tumor there are distinct clonal subpopulations of cancer cells with different patterns of genomic alterations [40]. Importantly, only a fraction of cells in the primary tumor were found to be genetically similar to other cancer cells collected in metastatic sites [40]. Thus, a complete genomic characterization of all subpopulations of cancer cells will be mandatory to identify the entire repertoire of cancer driver mutations. In line with this, Gerlinger and De Bruin [41, 42] proposed genomic/bioinformatic protocols for the in-depth molecular characterization of distinct subpopulation of cells. In this scenario, bioinformatics and cancer genomics are becoming key elements for the success of personalized medicine.

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